THE EFFECT OF NOXIOUS STIMULATION OF THE RIGHT MASSETER MUSCLE ON SINGLE MOTOR UNIT ACTIVITY AT TWO SITES IN THE MASSETER MUSCLE DURING STANDARDIZED JAW CLOSING TASKS

By

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DECLARATION

I hereby declare that the work described herein is, to the best of the knowledge, original and is entirely the work of the author, except where due acknowledgements have been made. The work was conducted while the author was pursuing a PhD degree program at the Faculty of Dentistry, the University of Sydney and carried out at the Jaw Function and Orofacial Pain Research Unit, Westmead Hospital under the supervision of Professor Greg Murray and Associate Professor Chris Peck. I certify that this thesis has not already been submitted, wholly or in part, for the award of the higher degree to any other university or institution and that all help received and all sources used in preparing this thesis have been acknowledged.

(Bushra Malik)

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In the name of Allah, the most Gracious, the most Merciful

"Truly strong is the Grip (and power) of the Lord.

It is He Who creates from very beginning, and He can restore (life).

And He is the Utmost-Forgiving, Full of loving kindness, Lord of throne of Glory,

Doer (without let) of all that He intends" Qur'an 85:12-15

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ABBREVIATIONS

APH: After hyperpolarization phase

ANOVA: Repeated-measures analysis of variance

ATP: Adenosine triphosphate

BK: Bradykinin

BL: Baseline session

CED: Cambridge electronic design

CGRP: Calcitonin gene related peptide

CNS: Central nervous system

CPG: Central pattern generator

DASS: Depression, Anxiety and Stress Scales

DC/TMD: Diagnostic criteria for temporomandibular disorders

EMG: Electromyography

FOG: Fast oxidative and glycolytic

FG: Fast glycolytic

Fint: Fast intermediate fatigable

FPL: Flexor pollicis longus muscle

FR: Fast resistant

HS: Hypertonic saline infusion session

IASP: International Association for the Study of Pain

IS: Isotonic saline infusion session

IPAM: Integrated Pain Adapatation Model

JFLS: Jaw functional limitation scale

LAT: Left anterior temporalis

LMAS: Left masseter

MHC: Myosin heavy chain isoform

MyLC: Myosin light chain isoform

MPQ: McGill pain questionnaire

MU: Motor unit

NRS: Numerical rating scale

NS: Nociceptive-specific neurons

PAM: Pain Adaptation Model

PCS: Pain Catastrophizing Scale

PGE2: Prostaglandin E2

PRI: Pain rating index

PTSD: Post traumatic stress disorder

RMI/RMP: Right masseter inferior/right masseter posterior

RMS/RMA: right masseter superior/right masseter anterior

RDC/TMD: Research diagnostic criteria for defining clinical subtypes of Temporomandibular Disorders

RMA: Right masseter anterior

RMI: Right masseter inferior

RMS: Right masseter superior

RMS: Root-mean-square

RMP: Right masseter posterior

SMU: Single motor unit

SO: Slow oxidative fibers

TMD: Temporomandibular disorders

TMJ: Temporomandibular joint

VAS: Visual analogue scale

VBSNC: Trigeminal brainstem sensory nuclear complex

VCT: Vicious Cycle Theory

WDR: Wide dynamic range neurons

PRESENTATIONS

A part of this thesis has been presented as oral and poster presentations in the following conferences.

Oral Presentation:

Bushra Malik, Terry Whittle, Greg M Murray. Recording of motor units during experimental muscle pain in masseter. Faculty Research Day, Faculty of Dentistry, The University of Sydney, Westmead, NSW, Australia, August 2014.

Poster Presentation:

Bushra Malik, Terry Whittle, Greg M Murray. Recording of single motor units at two different sites during experimental muscle pain in right masseter. Hospital week, Faculty of Dentistry, The University of Sydney, Westmead, NSW, Australia, June 2014.

ABSTRACT

The relationship between orofacial pain and jaw muscle activity has been thought to follow either *the Vicious Cycle Theory* or *The Pain Adaptation Model*. According to *the Vicious Cycle Theory*, there is a positive relationship between pain and muscle activity but this theory could not provide robust scientific evidence to support the model. On the other hand, *the Pain Adaptation Model* proposed an increase in muscle activity when the muscle was in the antagonistic phase and a decrease in muscle activity in the agonistic phase during muscle pain. The net effect would be slower and smaller jaw movements to protect the system from further pain and injury. Although the two theories have found support for the relationship between movement and the perception of pain, they have been unable to fully explain the effect of pain on muscle activity including the multidimensional nature of pain and individual and task related alterations in motor responses to pain.

More recent theories have been proposed, for example, the *Integrated Pain Adaptation Model* and the *New Theory on the Motor Adaptation to Pain*, which have attempted to explain the comprehensive organization of the jaw motor system along with psychosocial dimensions in order to understand the effects of pain on muscle activity. Numerous experimental pain studies have focused on the effects of experimental pain on jaw motor activity in the masseter muscle and have reported changes in surface EMG activity in association with tasks during pain. However, there is a little evidence to reveal details of changes in motor unit activity within the masseter muscle or to determine if motor unit

activity within the masseter muscle changes in response to noxious stimulation. There is a need to develop more clinically relevant models of jaw muscle pain which involves evoking pain through noxious stimulation and recording activity at more than one site within the masseter muscle.

An experimental muscle pain model that employs hypertonic saline infusion into the masseter muscle will provide a robust method to test suggested models. The addition of single motor unit activity recordings at two different sites within the masseter during the performance of isometric jaw-closing tasks in asymptomatic participants should provide an improved framework to understand the relationships between jaw muscle pain and motor behavior. The general aim of our study was to determine whether experimental masseter muscle pain resulted in a change in typical muscle activity at two different sites within the masseter muscle during the performance of isometric jaw-closing tasks in asymptomatic participants.

The specific aims of the present study were (1) to determine whether experimental masseter muscle pain alters the ability of individuals to perform isometric jaw-closing tasks; (2) to determine whether experimental masseter muscle pain leads to changes in recruitment patterns, thresholds of firing, or firing rates of single motor units, and changes in overall muscle activity, within the masseter muscle during standardized isometric jaw-closing tasks and these changes occur at 2 separate sites within the masseter muscle: (3) to determine whether any changes in recruitment patterns, thresholds of firing, or firing rates of single motor units, or changes in overall muscle

activity at one site within the masseter muscle during standardized isometric jaw-closing tasks are different to any changes occurring at another site within the muscle; (4) to determine whether experimental masseter muscle pain leads to changes in muscle activity at one or both sites within the masseter that are consistent with earlier theories of pain-motor interaction, namely, the *Vicious Cycle Theory* and the *Pain Adaptation Model;* (5) to explore associations between single motor unit characteristics and psychological measures.

To investigate the effect of experimental jaw muscle pain on muscle activity, single motor unit activity was simultaneously recorded with two bipolar fine wire electrodes placed at superior/anterior and inferior/posterior sites of the right masseter muscle during the performance of isometric jaw-closing tasks in 17 pain-free participants. Participants performed 3-5 trials of standardized biting onto an intra-oral bite force transducer during baseline recordings (without infusion), during experimental pain elicited in the right masseter with infusion of 5% hypertonic saline (pain), and during isotonic saline infusion (control). A within-subject design across different experimental blocks in the same participant reduced the between participant variability and enhanced the power of the study. Psychological dimensions of each participant were assessed through a number of questionnaires before and after the experimental recordings. Single motor units were identified and discriminated with Spike 2 (Cambridge Electronic Design Limited, Cambridge, UK) software during the ramp biting task and the step level biting task under baseline 1 and test sessions. Each unit that could be discriminated was analysed to determine the recruitment pattern across the recording blocks, the force level at threshold

of firing, the rate at which the single motor unit fired and the overall muscle activity using the root mean square of the electromyographic recording.

The results indicated that experimental masseter muscle pain induced by 5% hypertonic saline does not alter the ability of individuals to perform the isometric jaw-closing tasks. This finding is inconsistent with the *Pain Adaptation Model* which proposed a decrease in muscle activity in the closing force (agonist phase) during experimental muscle pain. For specific aim (2) and (3), the results showed that experimental masseter muscle pain led to changes in the recruitment patterns, but not the firing rates or thresholds of single motor units. Similarly, no changes were found in overall muscle activity within the masseter muscle during standardized isometric jaw-closing tasks. Changes in recruitment patterns of single motor units at one site within the masseter muscle during pain in comparison with control was observed during the standardized isometric jaw-closing tasks and some of these changes were different to the changes occurring at the other site within the same muscle. Inter-individual variability in the motor response to pain was observed in the present study during the standardized isometric jaw-closing tasks and between muscle activity at the two different sites of the masseter. This highly individualized motor response to pain is consistent with more recent models of pain-motor interactions which propose a comprehensive organization of jaw motor behavior to pain and is substantially variable between different individuals.

In support of the 4th specific aim, the findings provide support for a more complex model of motor adaptation to pain. The modified patterns of motor unit activity as a response to pain could be an attempt to maintain motor output to achieve a defined task and to protect the jaw motor system from further pain or injury. The data are not consistent with the

earlier models of pain-motor interactions, namely, the proposals of *the Pain Adaptation Model* and the proposals of *the Vicious Cycle Theory*.

For specific aim (5), no significant associations between psychological variables and jaw motor activity were found and this lack of an affect may be due to the low scores demonstrated by our physically healthy and psychologically well-functioning participants. This is in accordance with the findings of previous studies where experimental pain models were less likely to capture emotions and cognitions present in chronic pain patients.

An important finding from the present study was that the jaw motor system functions under pain in order to perform the particular jaw motor task. This finding provides support to more recent models proposing a re-organization of the recruitment strategy adopted by the brain in the control of motor units in the presence of pain. Our findings suggest that the sensorimotor system employs an altered motor unit recruitment strategy at different sites of heterogeneous muscle in order to perform particular jaw motor task. Further, inter-individual variability in hypertonic-saline-induced intramuscular effects on the standardized isometric jaw-closing tasks was also observed at two different sites within the masseter muscle in the present study. Taken as a whole, the findings of the present study appear to be more consistent with more recent models (*the Integrated Pain Adaptation Model* and *the Theory of Motor Adaptation to Pain*) which propose an individual's integrated motor response to pain varies with the complex organization of the sensory-motor system.

CHAPTER 1

REVIEW OF LITERATURE

1. INTRODUCTION AND OVERVIEW OF THESIS

The Global year against orofacial pain (2013-2014) sponsored by IASP brings global attention to pain that is perceived in the face and/or oral cavity and defines orofacial pain as pain caused by diseases or disorders of regional structures, by dysfunction of the nervous system, or through referral from distant sources. Common causes of orofacial pain are musculoskeletal (Temporomandibular Disorders (TMD), myofascial pain), neurological (trigeminal neuralgia, glossopharyngeal neuralgia, painful traumatic trigeminal neuropathy), neurovascular (migraine, trigeminal autonomic neuropathy) and psychogenic. According to population-based surveys conducted in different parts of world, the prevalence rate of pain in the orofacial region is around 18-26% (Macfarlane et al., 2002; John et al., 2003). The incidence and prevalence is higher in women than in men and generally the peak occurrence of symptoms is greatest among young to middle age adults than children and old subjects (Carlsson et al., 1999; Magnusson et al., 1999 Locker et al., 1991; Macfarlane et al., 2002).

Temporomandibular Disorders is a non-dental orofacial pain condition that mostly involves the temporomandibular joint (TMJ) and the associated muscles of mastication. The important signs and symptoms of TMD are pain and tenderness in and around the TMJ and the masticatory muscles, limitation of mandibular movements, and TMJ sounds.

TMD have a range of psychological impacts, and have a prevalence of 9-15% in women and 3-10% in men (Sessle 2005). The cause of the pain is unclear in some patients and if it persists, it can significant effects on the performance of sufferers to the extent that the pain can result in major disability such as extended sick leave and the sickness pension (Johansson1991). Other consequences of chronic TMD pain are mood changes, absenteeism, increased consumption of medications and reduced daily life activities.

Muscle pain has been considered to be the most common source of pain in patients presenting with chronic pain (Simon, 1998; Laat, 2001) and is often described as a dull aching sensation which is difficult to localize (Mense et al., 2001). Muscle pain is often referred from distant somatic structures involving the skeletal muscles, fasciae and/or tendons and may result from inflammatory (e.g. infection), traumatic (e.g. excessive opening of the jaw), or ischemic (e.g. through bruxing) factors and is frequently associated with changes to the sensitivity of superficial and deep tissues within the painful area (Graven-Neilson 2001, Travell 1982).

Chronic musculoskeletal pains tend to be more diffuse, persistent and resistant to quick or simple resolution than acute pains. The main problems involved in treating these kind of diseases is that they tend not to be well-defined diseases or there does not appear to be, in many cases, a well-defined lesion, and they chronic nature of these conditions can make diagnosis more difficult (Dworkin and LeResche 1992). Diagnosis is also complicated by the frequent presence of referred pain from muscle which may be associated with

hyperalgesia in somatic structures and the mechanism responsible for this may be central sensitization (see below) (Arendt-Neilsen et al 2001).

Past research (e.g. John D.Otis et al 2003) has demonstrated a strong association of stress and traumatic events with musculoskeletal pain even if the individual does not have obvious signs of a physical injury. Research indicates that patients with chronic pain related to trauma or PTSD (post-traumatic stress disorder) experience more intense pain and greater disability than pain patients without trauma (Sherman JJ et al., 2000). Previous experiences of traumatic events may become a source of traumatic memory, and may result in reflexive protective muscular activity escalating a cycle of pain (Alexander Cowell 2007). For example, it has been proposed that catastrophizing contributes to a fear of pain which may lead to avoidance of activities that elicit pain, guarding behaviours, and hypervigilance to bodily sensations (Vlaeyen et al., 2000; Akhter et al., 2014). Such avoidance may contribute to disability and depressive symptoms which further fuel the cycle of pain and fear and more avoidance.

1.1 Diagnosis of TMD

There is a large discrepancy between demand for seeking treatment and the actual need of treatment for TMD (Carlsson et al., 1999). TMD patients have been divided into 3 groups: active, passive, and no treatment need. “Active treatment need denotes patients with moderate or severe signs and symptoms of TMD that prompt the individual to seek help"; "Passive treatment need includes those with mild signs of TMD, perhaps no awareness of TMD with only minor or fluctuating symptoms". "No treatment need for TMD" refers to

those patients whose TMD problems did not call for treatment in any circumstances (Kuttila M, et a., 1998). The assessment of demand for seeking treatment is estimated as 1.5-30% whereas actual treatment need for the temporomandibular disorder is 2-5% (Carlsson 1999).

Literature has highlighted the need of future research to ascertain the etiological factors for TMD and the diagnosis of specific subtypes of TMD (LeResche 1997). There have been a number of diagnostic systems proposed for TMD and different systems use different criteria for clinical signs and symptoms. Nonetheless, even the most reliable and useful systems as proposed in the guidelines for classification, assessment and management by McNeil (McNeil et al., 1990) still need suitable criteria for evaluation (List and Dworkin., 1996).

The original RDC/TMD Axis II instruments were shown to be both reliable and valid (Schiffman, et al., 2014) although the Axis II protocol has been divided into screening and comprehensive self-report instrument sets. The screening instrument consists of 41 questions which assess pain intensity, pain related disability, psychological distress, jaw functional limitations, parafunctional behaviours and a pain drawing to assess the location of the pain. It assesses jaw functional limitations and psychological distress in detail as well as additional constructs of anxiety and presence of comorbid pain conditions (Schiffman, et al., 2014).

The recommended, evidence-based, new DC/TMD protocol is appropriate for use in both clinical and research settings. More comprehensive instruments augment short and simple screening instruments for Axis I and Axis II. These validated instruments allow for identification of patients with a range of simple to complex TMD presentations.

1.2 Management of TMD and the Need of Future Research

The levels of pain intensity and interference in psychological and psychosocial profiles in patients with TMD pain are similar to other pain conditions such as low back pain, headache and fibromyalgia (Forssell and Kalso 2004). The use of cognitive behaviour therapy in the management of TMD may be helpful for reducing pain intensity, limitation in jaw movement, and pain-related activity interference but the literature has shown little evidence (Liu et al., 2012).

Physical therapy has been recommended as a primary treatment option for patients with TMD and it has the general aim of alleviating pain, restoring normal joint function, reducing adverse loading of the masticatory system, and improving daily activities. The use of physical therapy, ultrasound, electrical stimulation, and thermal packs for musculoskeletal and TMD have been claimed to be useful, but there is little evidence to support their use (Feine and Lund 1997). More recent research has shown some evidence from basic science research to suggest that many of these therapies could have potentially therapeutic effects. However, there appears to be limited high-quality evidence from

randomized clinical trials to support the therapeutic effectiveness of several of the therapies (Al-Ani, et al., 2004; See review List et al., 2010).

Although the aetiology of TMD is still unclear, many predisposing, perpetuating and initiating factors have been identified. For many years, occlusal prematurities were considered to be important in the aetiology of TMD. However, recent evidence does not support a strong association between occlusal factors and TMD (Stapelman et al., 2008). Stabilization splints have been considered to be effective in reducing TMD pain in the short term compared to other treatment modalities such as physical and behavioural medicine, and acupuncture treatment. Overall, documentation on the long-term pain-relieving effect of occlusal appliances is limited, as it is for patient compliance in occlusal appliance treatment (List et al., 2010).

As the aetiology and the pathophysiology of craniofacial muscle pain are still unclear, there is an obvious need for continuing research in this field using evidenced-based study designs in order to improve the diagnosis and management of patients (Svensson and Graven-Nielsen 2001). Despite the recent research developments, the tendency to predominantly resort to traditional models to explain the diagnosis and management of orofacial pain is still ever-present. Recent research on neuroimaging of pain has led to new treatment approaches which will focus on extinction of aversive memories as well as restoration of normal brain function through methodologies such as brain stimulation and behavioural extinction training (Flor et al., 2012). As chronic pain appears to be a

transition from acute pain and as chronic pain appears to be associated with learning and memory related changes in central nervous system, new treatment methods should focus on alterations to central pain memories and maladaptive body perception (Flor et al., 2012).

1.3 Effect of Pain on Motor Activity and Need of Research

Many studies have been carried out to determine the effects of pain on electromyographic (EMG) activity in TMD patients (Glaros, Glass et al. 1997; Liu, Yamagata et al. 1999). One of the main aspects investigated in these studies has been to determine the effects of pain on muscle activity at the level of multi-unit EMG activity as would be acquired with surface EMG recording electrodes. As the single motor unit (SMU) has been demonstrated to be the basic functional unit of muscle, it has been considered that studies of the effects of pain on SMU activity may yield effects not demonstrable at the level of global muscle activity obtained from surface electrodes. (Sohn et al., 2000; Farina et al., 2001; Farina et al., 2004; Farina et al., 2005; See review Murray and Peck 2007) .

The effects of pain on the recruitment and firing rates of motor units have been mainly studied in the motor systems of the limb and trunk (Tucker et al. 2009; Tucker and Hodges 2009). In brief, these studies demonstrate complex effects of pain on SMU activity. By contrast, there is much less information available as to the effects of pain on the recruitment and firing rates of motor units in the jaw motor system, although there are some studies (Minami et al., 2013; Sohn et al., 2000; other Svensson et al., 1996; Miles et

al., 1987). The lack of information may be due to a range of factors including the difficulty of standardizing jaw tasks so that the effects of pain on motor unit activity can be studied between tasks performed under pain and no-pain conditions, as well as technical difficulties associated with the recording of single motor units in the jaw motor system. These recordings are technically difficult because fine wire electrodes are more difficult to place than surface electrodes as well as being painful to place compared to surface electrodes, and therefore subject recruitment is a major difficulty. The equipment needed to record single motor unit activity is also costly and requires specialized software to analyze the data.

A key question that has been addressed in many of these earlier studies in both trigeminal and spinal motor systems is whether the effects of pain on motor activity follow some of the previous theories explaining these effects. The Vicious Cycle Theory and the Pain Adaptation Model are 2 of the most widely cited theories that attempt to explain the relation between pain and motor activity. A number of recent studies, however, have indicated that neither of these earlier theories can fully account for the effects of pain in all classes of movement. These theories also cannot explain the maintenance of force if motoneuron discharge reduces in pain (for review, Murray and Peck 2007; Hodges and Tucker 2011). Given these and other issues related to these older models, new models have been proposed (Murray and Peck 2007; Hodges and Tucker 2011). One new model is called the integrated pain adaptation model (IPAM) which has proposed that the organization of the jaw motor system as well as the psychological dimensions of pain need to be considered in understanding the effects of pain on muscle activity.

As the individual's experience of pain varies widely, this newer model proposes the same occurs in an individual's motor response to pain, that is, the motor response to pain will vary between individuals depending on how the multi-dimensional nature of pain interacts with the motor system of an individual. There is also some evidence in the spinal motor system that pain may cause motor effects that may predispose to a recurrence of symptoms (Moseley et al., 2006; Hides et al., 1996; MacDonald et al., 2009). It may be necessary therefore to define how an individual's somatosensory and motor system operates while the individual is experiencing pain to allow a tailoring of management strategies to that individual. The IPAM encompasses the overall pain experience and its relationship to muscle recruitment strategies with the overarching purpose of minimizing pain and maintaining homeostasis. If, indeed, the Integrated Pain Adaptation Model does provide a basis for understanding the interaction between pain and motor activity, further research is needed to elucidate those pain-related variables that have an important influence on jaw muscle function.

It is well established that motor unit discharge rate is directly related to force production (Stuart and Enoka 1983). Other studies have also shown that, during noxious muscle stimulation, motor unit discharge rates decrease during contraction in the masseter muscle (Sohn et al., 2000; Thomas et al., 2000) and in the tibialis anterior muscle (Farina et al., 2005). More recent limb muscle studies have demonstrated that while some motor units become inactive during noxious stimulation, new motor units are recruited during knee extension tasks to assist in force maintenance (Tucker et al., 2009; Tucker and Hodges 2010, 2011).

Recent evidence in the jaw motor system has demonstrated that experimental painful stimulation of the masseter muscle results in both increases and decreases in motor unit activity within the masseter muscle during the generation of the same direction and level of force (Minami et al., 2013). This suggests that pain results in a reorganization of motor unit activity within the masseter muscle. This study (Minami et al., 2013) however only reported on the recruitment and the firing rates of single motor units at a single force level and at the same site within the masseter muscle. It is unclear whether this reorganization occurs at a number of different sites within the masseter muscle. It is also unclear whether the changes in single motor unit activity are observed at different force levels and whether there are changes in the threshold of onset of firing of single motor units. An understanding of these issues would add to our understanding of how motor unit activity within the masseter muscle changes in response to noxious stimulation. Further, it would help clarify whether re-organization of motor unit activity in response to noxious stimulation is a general feature of the jaw motor system in pain. This is important because some management strategies for chronic orofacial pain are based on prior simplistic models proposing uniform effects of pain on muscle activity (for review see, Murray and Peck 2007; Minami et al., 2013).

Given the limitations of previous EMG studies in patients with TMD, the inconsistencies between previous studies of the effects of pain on jaw motor activity, and the lack of information regarding the effect of pain on motor unit activity during standardized jaw tasks, the general aim of this study is to determine the effect of noxious masseter muscle stimulation on jaw motor unit recruitment and firing rate patterns during standardized jaw

tasks. This would be achieved by recording SMU activity during experimentally induced muscle pain at two different sites in the masseter. The following review of literature summarizes our current understanding of the pathophysiological mechanisms involved in jaw muscle pain, the information derived from human experimental pain models, physical properties and recruitment patterns of motor units and anatomical and functional information relating to the masticatory muscles, particularly the masseter muscle.

2. PAIN (DEFINATIONS AND TYPES)

The International Association for the Study of Pain (IASP) (1994) defines pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage." (Merskey and Bogduk 1994) . Although pain often has an obvious physical cause, many people describe pain in the absence of any obvious tissue damage or clear pathophysiological cause (Merskey and Bogduk 1994). Pain is unquestionably a sensation in a part or parts of the body, and it exhibits different qualities such as burning, tingling, sharp, pricking or aching.

Pain is an individual human experience that is entirely subjective and can only be truly treasured by the person experiencing the pain. It is always unpleasant and therefore this emotional experience is associated with actual or potential tissue damage.

Pain that is brief or short term and is sharp in nature is usually referred to as acute pain. This type of pain is evoked by intense heat or cold or intense mechanical stimulation, and serves as a diagnostic aid in determining the nature and site of the disturbance (Sessle

2000,Aghabeigi 2002). It is protective and acts as a warning of real or potential tissue damage that is manifest as a result of, inflammation, injury and operative procedures (Sessle B.J 2011). Acute pain occurs most commonly following injury or surgery and most patients can be treated effectively by simple analgesics. Effective timely treatment is essential to avoid the possibility of transition to chronic pain.

If acute pain is left untreated it may lead to the development of chronic pain which is difficult to diagnose and treat. It has been estimated that approximately 20% of acute pain conditions transition into chronic pain due to inappropriate management (Sessle B.J 2011). If the pain persists past the normal time of healing, which is generally considered to be 3 to 6 months, and then a painful condition is considered to be chronic (Mense et al., 2001). Chronic pain is often not affiliated with on-going tissue damage and a high level of discomfort may remain even when the initiating cause has been resolved (Okeson 1995). Chronic pain shows a different pathology (e.g. morphological, chemical and physiological changes in CNS) compared to acute which often worsens over time (Okeson 1995; Sessle BJ 2014). Chronic pain constitutes a serious, separate disease entity in its own right which if unrelieved, can have a devastating impact on all aspects of the sufferers' lives.

Chronic pain is a major health problem that imposes huge socioeconomic consequences including reduced quality of life and negative impact on relationships (Rice AS et al., 2015). Chronic pain has no clear biological role and if persists, it inflicts severe physical,

emotional and social stresses on both the persons involved and their families (Okeson 1995; (Sessle 2000); Racich 2005). The fact that many management strategies are largely ineffective is an important factor in the high incidence of depression (Sessle B.J 2011, 2012). In developing countries, several factors e.g. cost, government restrictions, and infrastructure of healthcare systems, act to limit the access of sufferers to receive the appropriate, timely care (Sessle B.J 2011; Rice AS et al., 2015).

It has been reported that musculoskeletal conditions have a significant impact on quality of life in terms of physical functioning or mobility and these conditions have been reported to be the most important causes of disability in members of the population in their employed years (Mense et al., 2001). Because of these limitations, musculoskeletal conditions are associated with significant socio-economic effects such as low education, loss of income (Johansson and Sojka 1991) and other psychological factors (Grassi et al., 2005) and has shown to be the strongest variable as a predictor in the development of depression (Magni G. et al., 1994).

Temporomandibular joint pain is called masticatory arthralgia as it arises from the joint and the ligaments (Okesson 1995; McNeill C 1990). In some individuals there may be a perceived alteration in the dental occlusion. Reviews of epidemiological studies have found that the prevalence ranges from 16% to 59% for reported TMD symptoms, and 33% to 86% for TMD clinical signs (Carlsson et al., 1999). A meta-analysis found a prevalence of 6% to 93% based on subject's reports and 0% to 93% based on clinical assessments (Carlsson et al., 1999, DE Kanter et al., 1993).

The limitations in previous assessment techniques for TMD has led to the development of the most reliable, evidence based system, the Research Diagnostic Criteria for Temporomandibular Disorders (RDC/TMD) by Dworkin and LeResche in 1992, and now more recently the Diagnostic Criteria for Temporomandibular Disorders (DC/TMD) by Schiffman et al. in 2014. The RDC/TMD is the most widely used system available to researchers and clinicians for the evaluation of subtypes of TMD. This is a dual axis system that allows physical diagnosis based on pathophysiology on one axis (axis I) and pain-related disability and psychological status on another axis (axis II) (Dworkin and LeResche 1992).

The RDC/TMD includes a history questionnaire and a clinical examination of operationally defined measurement criteria to diagnose the most common subtypes of TMD along with psychological assessment and specifications. Axis I has further sub-groups of muscle disorders, disc displacement, and a third group covering arthralgia, arthritis and arthrosis. These subgroups identify the structural and functional abnormalities of the muscles of mastication and the TMJs. Axis II on the other hand assesses and grades the severity of chronic pain intensity, pain related disability, depression and physical symptoms (Dworkin and LeResche 1992) which are important in evaluating and managing TMD pain. Axis II has greater importance in addressing psychosocial issues and have a significant influence on the patient's pain experience (Okeson 1997).

In a study of TMD patients employing the RDC/TMD (List et al., 1996), the diagnosis and clinical findings of TMD were compared and the study showed that 76% of patients had a muscle disorder, 32% to 39% had a disc displacement disorder, and 25% to 32%

had arthralgia, arthritis, or arthrosis disorder in the right and/or left joints. A psychological assessment showed 18% exhibited depression and 28% had non-specific physical symptoms (List et al., 1996).

The original Research Diagnostic Criteria for Temporomandibular Disorders (RDC/ TMD) Axis I diagnostic algorithms have been demonstrated to be reliable (Eric Schiffman et al., 2014). However, its validity was below the target sensitivity and specificity (Eric Schiffman et al., 2014) which supported the development of the revised RDC/TMD Axis I diagnostic algorithms which have been demonstrated to be valid for the most common pain related TMD. Diagnostic criteria for temporomandibular disorders (DC/TMD) include both a valid screener for detecting any pain-related TMD as well as valid diagnostic criteria for differentiating the most common pain-related TMD and intra-articular disorders.

Chronic pain has a detrimental effect on physical and psychological health (Crook J, 1984, Gureje et al., 1998, Becker Neils 1997, Latham 1994), routine activities (Bowsher D 1991), employment (Latham 1994) and economic well-being (Locker D 1983). Although there have been remarkable advancements in understanding the mechanisms of chronic pain, as well as the need for improved diagnosis and treatment, a considerable gap in knowledge of these issues still exists. Further, management strategies need to be adapted to each country in order to enhance the awareness of orofacial pain given the socioeconomic and educational situation within that country (Sessle B.J 2011).

3. PATHOPHYSIOLOGY AND MECHANISMS OF PAIN

The following review will give an overview of the pathophysiology of pain and will highlight, where appropriate, those aspects of particular relevance to pain from muscles. Orofacial pain, whether originating from the skin, mucosa, the temporomandibular joint (TMJ), or musculature, arises because of nociceptive activity in a range of peripheral and central pathways. We can experience orofacial pain when four distinct processes occur and these are known as neural transduction, neural transmission, neural modulation and perception. The pathophysiology and main mechanisms of TMD pain mainly involve the trigeminal nerve as the principle nerve carrying nociceptive information into the brain and most of the primary sensory afferent fibres have their cell bodies within the trigeminal ganglion (Sessle B.J 1999). It has been recognised that the somatosensory afferent information from the orofacial region to second order neurons in the brainstem not only travels via the trigeminal nerve but also via the roots of the facial, glossopharyngeal, vagus and upper cervical nerves (C1-C3) and all these nerves play a part in conveying input to higher centres (Aghabeigi 2002).

Free nerve endings provide the peripheral basis for transduction of noxious stimuli into action potentials and are present in all tissues of the orofacial region including skin, oral mucosa, dental pulp, periodontium, TMJ and jaw muscles. Peripheral stimulation through noxious substances causes excitation of nociceptors which once activated can undergo peripheral sensitization processes that are important in the allodynia and hyperalgesia noted in many orofacial pain states (Hannam and Sessle 1994; Sessle B.J 2001). When

the nociceptive terminals are activated, action potentials travel towards the brain for further processing.

Table I-1 classifications of peripheral afferent nerve fibres (From Dubner 1978)

Type of nerve fibre	Information carried	Myelin sheath?	Diameter (micrometers)	Conduction speed (m/s)
A-alpha	proprioception	myelinated	13-20	80-120
A-beta	touch	myelinated	6-12	35-90
A-delta	Pain (mechanical and thermal)	myelinated	1-5	5-40
C	Pain (mechanical, thermal and chemical)	Un-myelinated	0.2-1.5	0.5-2

The nociceptive fibers are divided into three main types as A-δ, C-fiber and silent or sleeping nociceptors. A-δ fibers are further divided into small diameter (2-5 μm), myelinated, fast conducting (5-30 m/s) fibers which respond mainly to noxious mechanical stimuli (Lynn B, 1994), and larger diameter fibers which respond to stimuli of all types i.e. mechanical, chemical and thermal stimuli, and conduct impulses from the skin and mucosa, as well as muscles and joints. However, C-fibers are unmyelinated, have a smaller diameter of 0.2-1.5 μm, a slower conduction velocity (0.5-2 m/s) and many respond to noxious mechanical, thermal and chemical stimuli (Table I-1). Both A-δ and C-fibers terminate in peripheral tissues as free nerve endings innervating all orofacial tissues and where they provide the peripheral basis for temperature and pain (Sessle B.J

1999; Sessle B.J 2006). Silent nociceptors are insensitive and become active only after tissue damage and add in nociceptive input to CNS (Aghabeigi 2002).

Activation of the nociceptors causes the generation of action potentials in their associated afferent fibres, and the signals are then conducted into the CNS, where the nociceptive information specifically terminates in the subnucleus caudalis of the trigeminal brainstem sensory nuclear complex or medullary dorsal horn. This provides the brain with sensory-discriminative information leading to the perceptual, reflex and other behavioural responses characterizing pain (Lund and Sessle 1994; Svensson et al., 2004; Sessle B.J 2000). Tissue trauma, inflammatory conditions or peripheral nerve injuries cause release of chemicals (e.g. K+, prostaglandins and bradykinins) that change the properties of peripheral nociceptors, and thereby lead to a sensitization of nociceptive afferent endings (Sessle B.J 2006). The resultant hyperexcitability is called “peripheral sensitization”. Peripheral sensitization is also a factor in the spread and referral of pain which is a characteristic of pain in the TMJ and/or associated musculature as seen in TMD.

Other stimulants namely, adenosine triphosphate (ATP), protons (leading to a reduction in tissue pH), inflammatory cytokines (interlukins), and neurotrophins (nerve growth factor), can increase the excitability of nociceptive afferent endings. Receptor molecules for these substances can be found in dorsal root ganglion cells of muscle afferent fibers. Studies using in vivo electophysiological, and in vitro recordings involving molecular and immunocytochemical approaches, have shown that trigeminal ganglion cell bodies

and their counterparts in the dorsal root ganglion synthesize chemicals that help in defining the role of primary afferent nociceptors in encoding noxious stimuli (Turp et al., 2007).

Further research is needed to understand the role of neurochemical and molecular markers of nociceptive afferents supplying orofacial tissues and their termination patterns in different compartments of trigeminal brainstem sensory nuclear complex (VBSNC). The trigeminal brainstem sensory nuclear complex (VBSNC) is divided into the primary or main sensory nucleus and the trigeminal spinal tract nucleus (Figure I-1).

The main sensory trigeminal nucleus is rostrally located and receives low threshold input from many orofacial tissues including periodontal, TMJ and muscles. The trigeminal spinal tract nucleus on the other hand is caudally located and is subdivided into subnucleus oralis, subnucleus interpolaris and subnucleus caudalis.

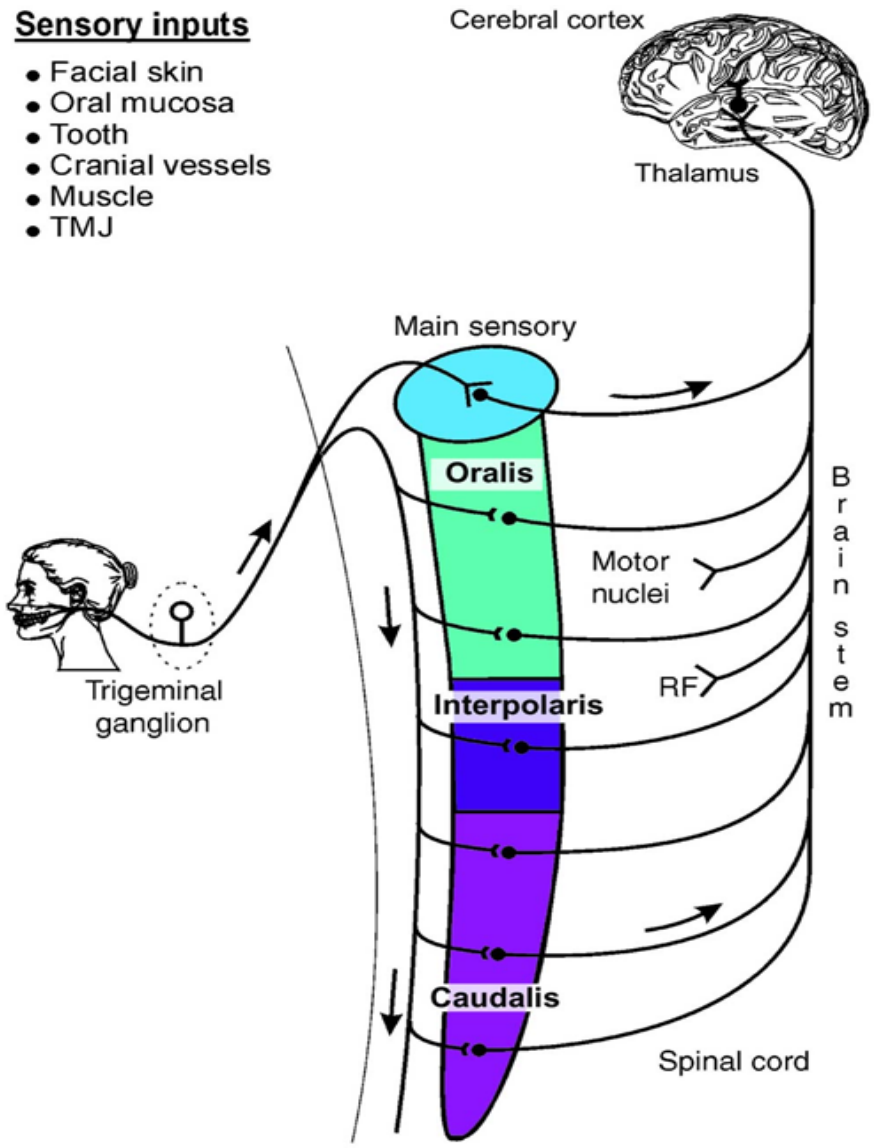


Figure I-1 The spinal tract nucleus pathways whereby trigeminal somatosensory information enters the brain can be subdivided from rostral to caudal into subnucleus oralis, interpolaris and caudalis (From Sessle B.J 2011).

There is evidence that subnucleus caudalis receives most of the nociceptive information travelling in the trigeminal system and has a close structural and functional relationship with the spinal region (Sessle B.J 1999). Subnucleus caudalis is a laminated structure that extends into the spinal cord and merges with the dorsal horn of the spinal cord.

Nociceptive primary afferents terminate in the superficial and deep laminae of the subnucleus caudalis which serves as a relay station for nociceptive information from the orofacial region. Evidence suggests that subnucleus caudalis is homologous to the substantia gelatinosa of the spinal dorsal horn by acting as gate mechanism capable of modulating somatosensory information from facial skin and deep tissues and plays a crucial role in the perception and localization of acute superficial and deep orofacial pain (Dubner et al., 1978; for review, see Türp et al., 2007; Sessle B.J 2011, Aghabeigi 2002).The rostral component on the other hand, that is, the subnucleus oralis, represents important element in orofacial pain and is mainly involved in intraoral and perioral pain mechanisms (Hannam and Sessle 1994; Sessle B.J 2006).

The superficial (lamina I) and deep (lamina V) laminae of the subnucleus caudalis contain second order neurons which are activated by a range of noxious stimuli. On the basis of their mechanoreceptive properties, these second order neurones have been characterized as either nociceptive specific (NS) or wide dynamic range (WDR). The NS neurons receive input from small diameter A-δ and C-fibers and respond only to noxious mechanical and thermal stimuli applied to the receptive fields of neurons of the face and mouth region. The WDR neurons in contrast, receive input from large diameter afferents, e.g. from A-β fibers, and also from nociceptive primary afferents. These WDR neurones respond therefore to low-threshold non-noxious stimuli as well as noxious stimuli. In general, the WDR neurones have large receptive field sizes compared to those of the nociceptive specific (NS) second order neurons. However, both types of nociceptive

neurones provide information about the intensity and location of a noxious stimulus (Lund and Sessle 1994).

Evidence suggests that electrical, algesic chemical or natural (intense pressure) stimulation activates various small diameter primary afferent nociceptive fibers supplying the TMJ, jaw and tongue muscles. This nociceptive activity then causes excitation of around 60% of the WDR and NS neurones. This information then relays to 3rd order neurons in the thalamus and higher regions including the cerebral cortex (Hannam and Sessle 1994).

It is necessary to explain the phenomena of convergence and divergence before illuminating the mechanism of referral of pain. When primary afferent neurons enter into the central nervous system they synapse with second order neurons which carry the impulses to higher centres. When several primary afferent neurons synapse with one second order neurone, neural information travelling along these separate primary afferents is said to converge onto the single second order neuron. This phenomenon is called “convergence”, and “divergence” occurs when single primary sensory neurons branch to synapse with several second order neurones. Many nociceptive neurons receive afferent input from other cranial and cervical nerves. Different convergence patterns of NS and WDR neurons of caudalis and afferent input from musculoskeletal, tooth pulp and mucosal tissues together with central sensitization has associated in diffuse and referral pain which is the feature of orofacial pain.

The nociceptive information from the trigeminal sensory nucleus and subnucleus caudalis partly projects to the thalamus and a portion of the projection ends in reticular formation and adjacent brainstem areas. The thalamic regions entirely involved in receiving and relying nociceptive information are the ventrobasal complex or the ventroposterior complex and the medial thalamus (Hannam and Sessle 1994).To distinguish the sensory aspects of pain, it is thought that the WDR and NS neurons in the ventrobasal thalamus project directly to the primary and secondary somatosensory cortices, and their main role is thought to be in the localization and discrimination of pain. On the other hand, neurons associated with the medial thalamus and conveying nociceptive information generally have an extensive receptive field and are thought to play a central role in the affective or motivational dimensions of pain.

Recent evidence has shown that the caudal part of subnucleus caudalis merges with the cervical dorsal horn, while the rostral part forms a ventral transition region which receives a bilateral afferent input from the orofacial tissues (Turp et al 2007). Persistent peripheral nociceptive input may lead to neuroplastic changes in central nociceptive neurons. These neuroplastic changes consist of a cascade of chemical events that brings about changes in the properties of the second order and higher nociceptive neurones. These changes include expansion of receptive field sizes, lowering of activation thresholds and enhancement of responses to craniofacial stimuli. These changes in nociceptive neurons may persist for long period of time depending upon the nature and type of injury and this neural change in processing at higher centres is termed functional “neuroplasticity” or “central sensitization” (Sessle B.J 1999, Lund and Sessle 1994).

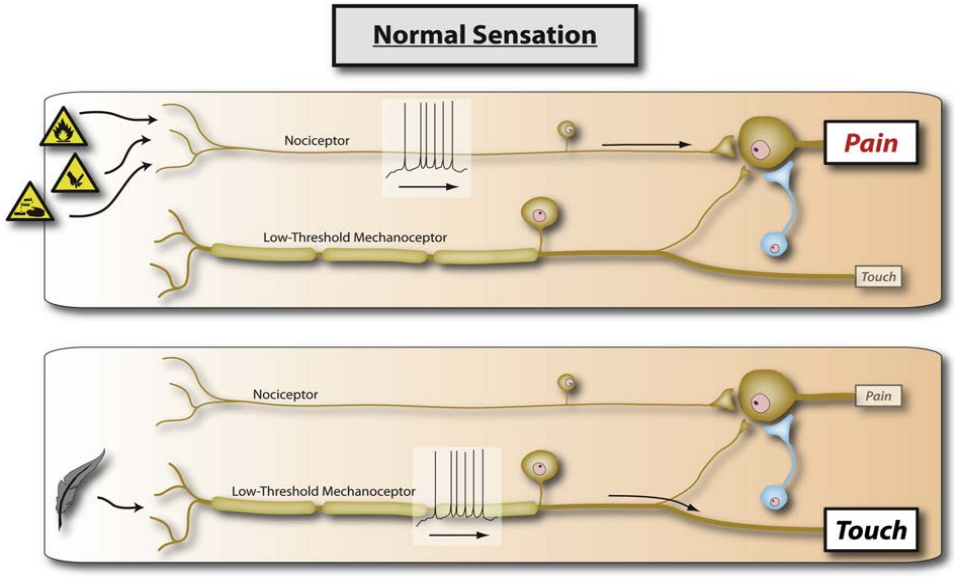


Figure I-2 Normal sensation. The somatosensory system is organized such that the highly specialized primary sensory neurons that encode low intensity stimuli only activate those central pathways that lead to innocuous sensations, while high intensity stimuli that activate nociceptors only activate the central pathways that lead to pain and the neurons that focus activity to these dedicated circuits (from Woolf et al., 2011).

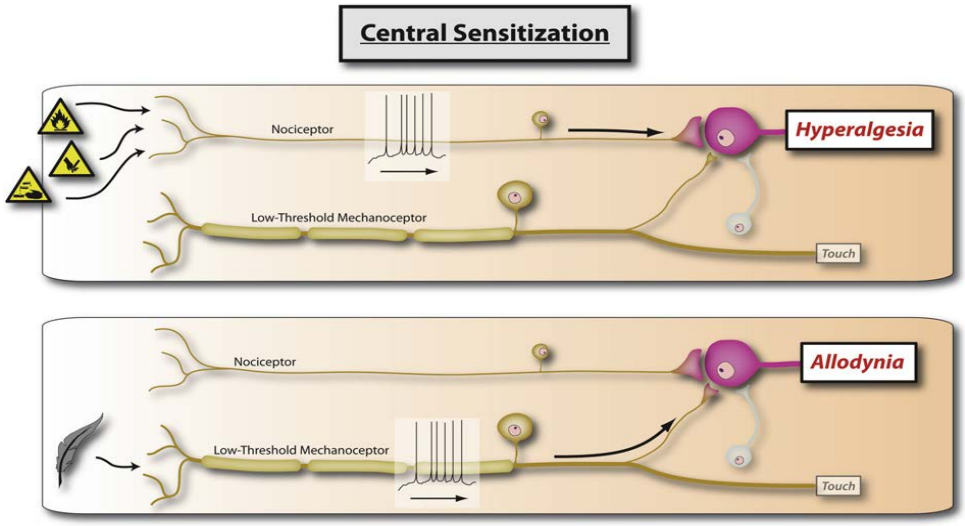


Figure I-3 Central sensitization. With the induction of central sensitization in somatosensory pathways with increases in synaptic efficacy and reductions in inhibition, a central amplification occurs enhancing the pain response to noxious stimuli in amplitude, duration and spatial extent, while the strengthening of normally ineffective synapses recruits subliminal inputs such that inputs in low threshold sensory inputs can now activate the pain circuit. The two parallel sensory pathways converge (From Woolf et al., 2011).

It has been noted that excessive depolarization of nociceptive neurons resulting from enhanced nociceptive input from an injured site, promotes excitatory mechanisms and in some individuals, a failure of endogenous inhibitory mechanisms both of which collectively may contribute to prolonged pain (for reviews, see Dubner,1997; Sessle B.J 2006; Ronald Dubner et al., 2014).These findings of central sensitization highlight the fact that the nociceptive pathway is not unyielding and inflexible but rather can undergo modification at the peripheral level and these neuroplastic changes can continue in central neurons (Figure I-2 and I-3). One of the main goals of these changes is that of alerting the organism to damage and to ensure that the organism protects the injured tissue from further injury.

Recent literature shows that the development of the concept of central sensitization has conceptually ‘‘centralized” pain and raised awareness of the importance of central changes in the brain as being important in the maintenance of particularly the chronic pain state, instead of it being thought to be exclusively peripherally driven. A noxious stimulus can induce pain and the amplitude and duration of pain correlates with the magnitude of the noxious stimulus. In the absence of significant tissue damage, nociceptive pain is determined solely by the intensity and timing of these nociceptive inputs. It was therefore believed that in the absence of such potentially damaging stimuli there should be no pain (Woolf et al., 2009). However, after the discovery of central sensitization it became clear that a noxious stimulus was not necessary to produce pain. Further, it was found that if the gain of neurons in the ‘‘pain pathway” in the CNS was increased, then low threshold, innocuous inputs can activate the “pain pathway”. Such

pain has no longer been termed as nociceptive only, but rather reflects a state of induced pain hypersensitivity, with almost precisely the same ‘‘symptom” profile to that found in many clinical conditions.

There have been many studies in human asymptomatic volunteers and patients, as to the role of central sensitization, defined operationally as an amplification of neural signaling within the CNS that produces pain hypersensitivity, and its relative contribution to inflammatory, neuropathic and dysfunctional pain disorders (Woolf et al., 2009, Costigan et al., 2009, Woolf et al., 2004). It is becoming clear from human and animal experimental studies that orofacial pain is associated with changes not only in the brainstem sensory and motor circuits but also at higher centers of the brain which contribute to the sensory (e.g. allodynia and hyperalgesia) and motor (e.g. altered muscle activities) effects seen in orofacial pain states (Sessle B.J 2014).

The mechanisms of peripheral and central sensitization, and the features of convergence and divergence of somatosensory information in the brainstem, help to explain the possibility of the persistence of pain even in absence of a continued peripheral stimulus, as well as the poor localization and spread of pain. Although significant work has been done that has helped us to understand the persistence of pain in the absence of any stimulus or abnormality, further research is still needed to understand how nociceptive information from the orofacial region (TMJ and musculature) is being processed in the thalamus and cortical regions. The relative role of the rostral and caudal components of

the VBSNC in the nociceptive responses to noxious stimulation of cutaneous and deep craniofacial tissues represents an important subject that requires future research. There are also sex differences in the responsiveness of nociceptive primary afferents, and it is possible that these differences may contribute to the sex differences documented for many orofacial pain conditions (Turp et al 2007).

4. EXPERIMENTALLY INDUCED MUSCLE PAIN

Many studies have been carried out in experimental animals to study the transmission, processing and effects of nociceptive activity following noxious stimulation of the orofacial tissues (for review, Sessle B.J 2000). Given the many differences between animals in comparison with humans, it is difficult to extrapolate the experimental data from animal studies to the clinical condition of muscle pain. Therefore, experimental muscle studies have been devised in human volunteers with the intention of simulating the pain experienced by patients with chronic muscle pain (Graven-Nielsen and Mense 2001). With regards to the study of chronic pain patients, another issue to consider is that it is difficult or even impossible to study cause-effect relationships of disease or pain with clinical research given that the patient already has the condition. Therefore human experimental pain research has been developed to help in obtaining more reliable knowledge of the mechanisms involved in pain transduction, perception and transmission under normal and pathological condition (Arendt-Nielsen and Svensson 2001).

Several approaches have been used in experimental studies of asymptomatic volunteers which allow a comparison of “before” and “after” conditions or to a “nonpainful” condition. Different types of experimental models have been used, namely exogenous models, endogenous models or a combination of both. In the exogenous pain model, pain is produced by the application of nociceptive mechanical (intense pressure), chemical (hypertonic saline, glutamate, serotonin, capsaicin and substance P) or electrical (in TMJ capsule) stimuli to a muscle or a joint. Endogenous models on the other hand, produce pain by endogenous release of chemicals such as bradykinin and prostaglandins which directly activate muscle nociceptors. The combination of both exogenous and endogenous models (e.g. ischemic block of superficial temporal artery with temporalis muscle exercise) produces pain more quickly.

The method of intramuscular infusion of hypertonic saline (6%) was first introduced by Kellgren and Lewis in 1938 (Kellgren 1938; Lewis 1938). This method has been extensively used since then because the quality of the induced pain is comparable to an acute episode of clinical muscle pain. It is safe, easy to use, and it induces local and referred muscle pain by activation of nociceptors in around 40-85% of individuals (Arendt-Nielsen and Svensson 2001). Experimental studies have shown that chemical and mechanical stimuli can cause excitation of muscle nociceptors and thus leading to a nociceptive afferent response and thereby resulting in muscle pain and hyperalgesia in humans.

Human experimental and clinical pain studies have increased our knowledge in understanding the mechanism of pain and the other factors (e.g. genetic and environment risk factors) involved in orofacial pain. Human experimental pain research provides evidence that both peripheral and central sensitization mechanisms play important roles in the pathophysiology of pain and studies are emerging that nociceptors have complex effects on the motor system (Ervilha U.F. et al., 2005; Sessle B.J 2014). More research is still needed to gain a deeper insight about peripheral and central mechanisms involved in the pathophysiology of pain. Similarly, research on other factors effecting orofacial pain such as genetic and environmental risk factors should allow us to better understand the mechanisms involved and will provide knowledge to construct better diagnostic, preventive and curative (pharmacological and non-pharmacological) methods of chronic orofacial pain

5. THE SINGLE MOTOR UNIT

The single motor unit is considered to be the basic functional unit of a muscle that can be recruited and controlled by the central nervous system. It consists of an α-motoneuron and a set of muscle fibers innervated by that neuron. Each muscle fiber is innervated by only one motoneuron, and each motoneuron can innervate from ten to thousands of muscle fibers. Jaw muscle motor units contain several hundred muscle fibres. The excitation of a motoneurone and the resultant discharge of action potentials cause an activation of muscle fibers which in turns produces muscle contraction and force generation. This whole complex of nerve cell, the nerve fibre and its terminal branches, the neuromuscular junctions, the muscle fibres and its constituent myofibrils, is the

pathway through which nervous activity gives rise to voluntary movements (Lenman et al., 1987).

5.1 Physiology of Motor Units

Motor units possess several different properties. Identification of the motor unit was a major discovery in our understanding of how the central nervous system (CNS) controls motor output (see Clarac and Barbara, 2011; Stuart and Brownstone, 2011). The ability of a motor unit to produce force depends upon the cross sectional area of the constituent muscle fibers whereas the speed of contraction of a motor unit is dependent on heavy-chain of the myosin protein in the muscle fibers. The size of a motor unit in a muscle is known by the innervation ratio, which is the ratio between the number of muscle fibres in a muscle and the number of motor nerves supplying it.

The single motor unit has been extensively studied from different approaches including studies of the anatomy, histology, and physiology, in an attempt to clarify its function and it has been found that MU's show a great variability in morphology, physiology and biochemical properties (Turkawski and Van Ejiden 1998; Review Burke R.E. 1986). Most information on the physiological properties of single motor units comes from animal experiments involving stimulation of motoneurons directly by insertion of electrodes into the motor nucleus supplying the limb muscles (Frend H.J 1983; William R. et al 1978; Kanda k, et al., 1977).

Initially, muscle fibers were classified into type I (slow) and type II (fast) fibers and this classification was based on the ATPase activity of muscle fibers in alkaline (Guth and Samaha,1970) and acidic media (Brooke and Kaiser,1970). Later on, type II fibers were subdivided into IIA and IIB and some new fiber types (IIX, IIC and IM) were also identified by this method. Based on enzyme histochemistry, muscle fibers can be classified into slow, oxidative (SO) fibers, recruited for slow postural activity and fast, either oxidative and glycolytic (FOG) or glycolytic (FG), for fast contractions (Barnard et al., 1971; Burke et al., 1971, Peter et al., 1972). Motor units have also been classified into slow motor units and fast motor units. Fast motor units are further classified into FR (fast, fatigue resistant), Fint (fast intermediate fatigable), and FF (fast fatigable) (Burke et al., 1967; Burke et al., 1973; Burke et al., 1974).

A large number of differences exist when comparing the physiological properties of jaw muscles with the muscles of limbs and trunk. A range of different properties of masseter motor units has been discussed and compared with the motor units of the limb and trunk muscles (Van Eijden and Turkawski 2001). It was found that masticatory muscle motor units appear to be smaller than motor units of the limb and trunk muscles and also appear to show a more localized organization of motor control which permits the differential control of separate muscle portions. There are other differences also. Masticatory muscle fibers express Myosin heavy chain isoform that is not expressed in adult trunk and limb muscles (Butler-Browne et al., 1988; Bredman et al., 1991; d'Albis et al., 1991; Stal et al., 1994). The presence of MHC (myosin heavy chain) isoform results in a high range of contraction speeds in masticatory muscle motor units. In jaw muscles, type-I fibers are

larger than type-II, while the opposite is true in limb and trunk muscles (Polgar et al., 1973). Similarly, it has been reported that type-I fibers in masseter are slower than the same fiber types of the limb and trunk muscles (Morris et al., 2001).

Along with this, variability in contractile properties also exists and it has been found that type-I masseter muscle fibers shows larger variability when compared to limb and trunk muscles. This difference might be related to difference in the expression of the light chain of myosin (MyLC). In the masseter muscle, 4 different MyLC's (MyLC-1, MyLC-2, MyLC-1f, MyLC-lemb/atrial) are expressed whereas MyLC-2f and MyLC-3f has been expressed in limb and trunk muscles which are not found in the masseter muscle (Soussi-Yanicostas et al., 1990; Stal et al., 1994). Furthermore, differences in cross sectional area of the muscle fibers between limb and masticatory muscles have also been observed. Generally, jaw muscle fibers are smaller in cross sectional area compared to the large cross section areas of limb and trunk muscles.

The literature also shows that many differences exist between and within the jaw muscles and a comparison of jaw opening muscles with jaw closing muscles shows that jaw openers are simpler with respect to activation, architecture and fiber type composition than the jaw closing muscles (Tsuruyama et al., 2002; Korfage et al., 2000). It was found that fibers of the jaw closers express 70% MyHC-I (See Figure 1-4) compared to the jaw openers which showed MyHC-I was expressed in 40-45% of all fibers. On the other hand, jaw closers have only few fibers that express MyHC-II (30%) than jaw openers (50%).

Furthermore, jaw closers contain 40% of hybride muscle fibers compared to only 10% fibers of jaw openers (Korfage et al., 2001).

Differences also exist between the muscles of a particular group, e.g. temporalis contains more MyHC type I and IIA fibers and few hybrid fibers than seen in the masseter and medial pterygoid muscles (Korfage et al., 2000). Heterogeneity in fiber type composition was also observed particularly in the jaw closing muscles and may reflect stretch related fiber type adaptation or there may be a genetic basis to this heterogeneity within a muscle. A good example of heterogeneity within a muscle is in the masseter muscle, in which the anterior and deep portion contains more type I fibres than the posterior and superficial part. It has been observed with EMG recordings from the masseter muscle with fine wire electrodes (Blanksma et al., 1997) that the anterior portion was activated more frequently than the posterior. It was speculated that the anterior portion of the masseter was more frequently activated and this might lead to a higher portion of MyHC type I fibers than the posterior part. Slow motor units are larger in deep and anterior region of masseter muscle responsible for fine control of muscle force and resistance to fatigue during chewing and biting (Van Eijden et al.,1993), whereas Fast motor units are larger in superficial and posterior part.

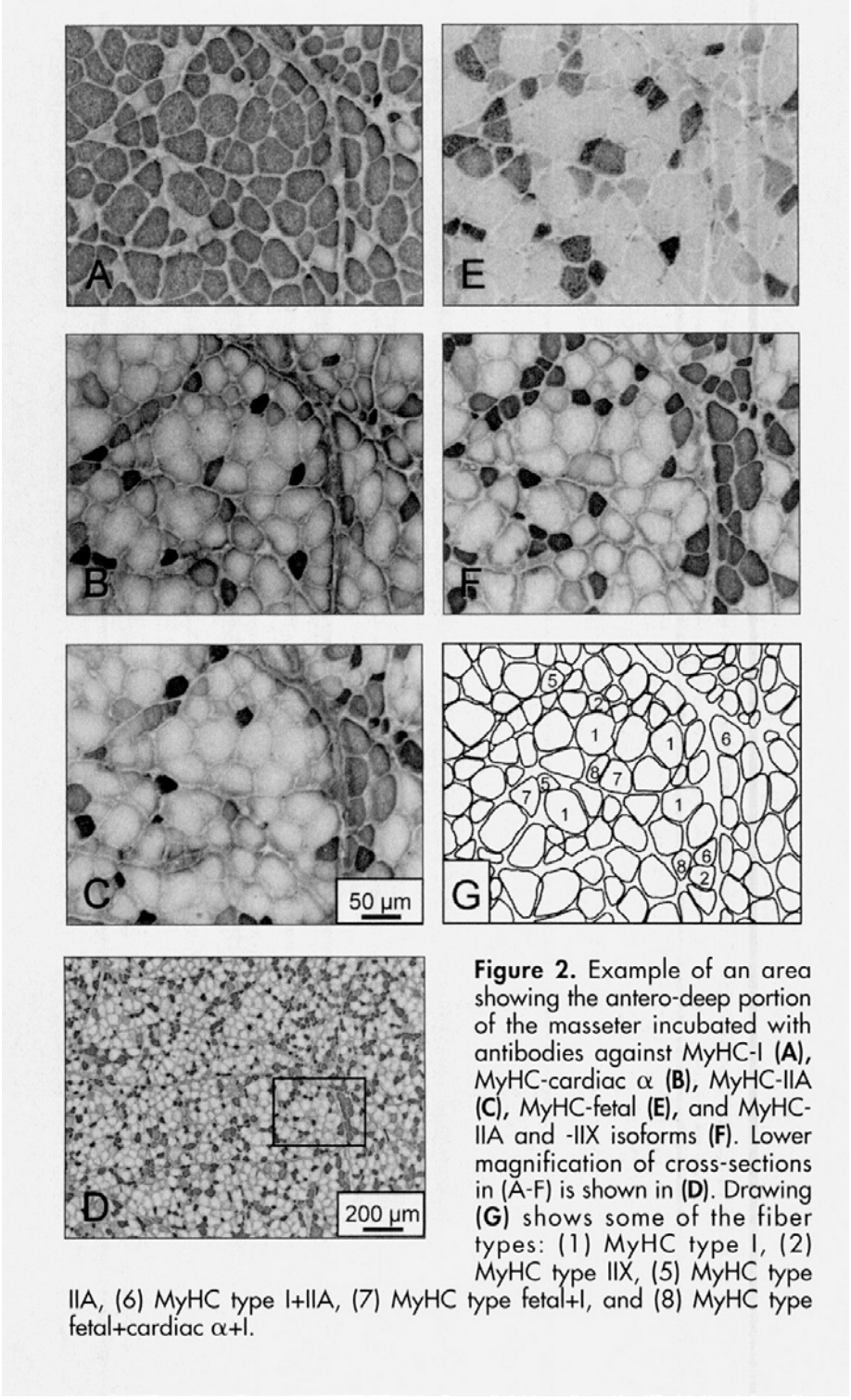


Figure 2. Example of an area showing the antero-deep portion of the masseter incubated with antibodies against MyHC-I **(A)**, MyHC-cardiac α **(B)**, MyHC-IIA **(C)**, MyHC-fetal **(E)**, and MyHC-IIA and -IIX isoforms **(F)**. Lower magnification of cross-sections in (A-F) is shown in **(D)**. Drawing **(G)** shows some of the fiber types: (1) MyHC type I, (2) MyHC type IIX, (5) MyHC type IIA, (6) MyHC type I+IIA, (7) MyHC type fetal+I, and (8) MyHC type fetal+cardiac α+I.

Figure I-4 An area of antero-deep portion of masseter incubated against antibodies against MHC-I (from Korgage et al Fiber type part-1 2005)

It was found that when the jaw is opened, those muscle portions with a long moment arm, such as the anterior portions of the masseter and the temporalis, undergo large sarcomere excursions, and are subjected to more stretch than the posterior portions which have a shorter moment arm (Van Eijiden and Raadsheer, 1992; Van Eijden et al., 1996). These different physiological properties of motor units in the human jaw muscles appear to be an important requirement of the human jaw motor system that has to perform a variety of tasks including biting and mastication of different kinds of foods, speech, singing, swallowing and yawning.

5.1.1 Motor Unit Territories

Initially, phosphorylase and glycogen depletion methods have been used to describe motor unit territories in animals by Kugelberg and Edstrom (Edstrom et al., 1968) They investigated the functional organization of different types of fibers and mapping of motor units in limb muscles of the rat and cat and disclosed a wide range of contraction, speed, tension output and fatigability. In these histochemical studies, the contraction of muscles produce striking changes in phosphorylase activity and glycogen contents in different types of muscle fibers and these chemical changes bear a relationship to muscle fatigue and thereby reflect metabolic differences in different fibers on activation (Kugelberg and Edstrom, 1968). These studies have shown that the muscle fibers of motor units are intermixed with each other and are restricted to a particular region of the muscle, known as, the motor unit territory See Figure 1-5). Within the territory of a motor unit, 15-30 fibers of other motor units can be found (Buchthal and Schmalbruch, 1980; Herring et al.,

1989). Fig I.5 gives an example of these histochemical studies showing the distribution of the fibres of single motor units.

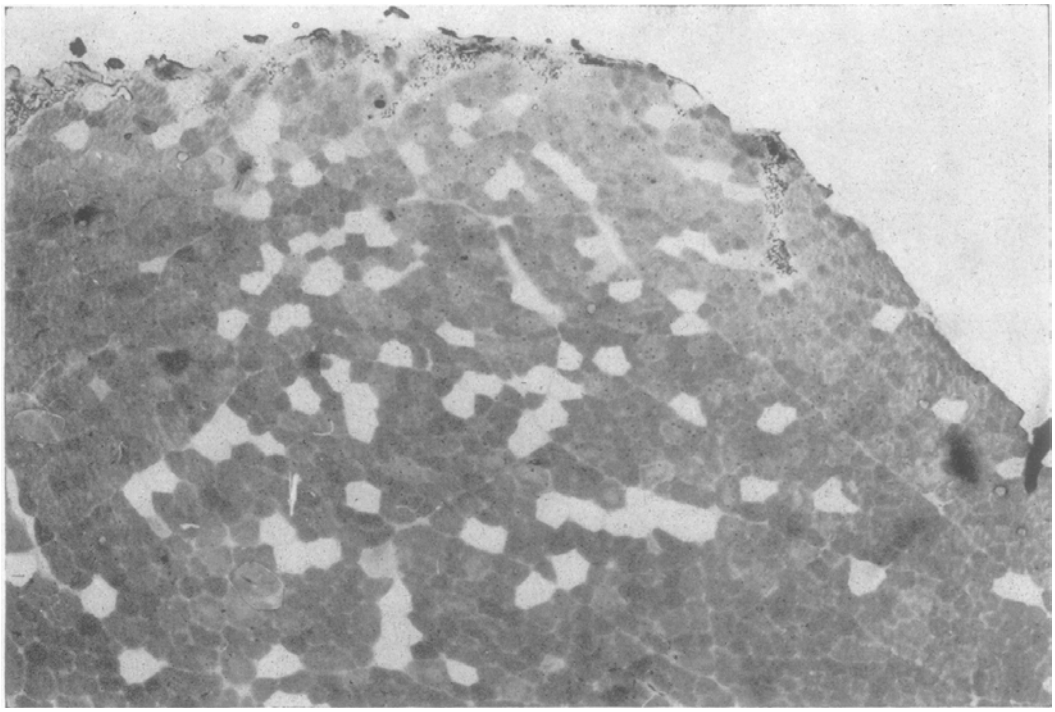


Figure I-5 Cross-section showing the distribution of the fibres of single motor units which were PAS negative. (From Lars Edstrom and Eric Kugelberg 1968).

Later on, a technique of electrophysiological cross-sectional scanning of motor units in human muscles was developed by Stalberg and Antoni initially in 1980 and later on by Stalberg and Eriksson in 1987. This initial method gave information about the organization of individual muscle fibers and the propagation of action potentials within the motor units. The width of the motor unit territory in the human masseter muscle was estimated by Stalberg and Eriksson (1987) and McMillan and Hannam (1991) using an EMG scanning technique and it was reported that motor unit territories are small and occupy only 5% of the muscle cross-sectional area compared to the limb and trunk

muscles where motor unit territories occupy 10-15% of muscles' cross-sectional areas ((Edstrom and Kugelberg, 1968; Burke and Tsairis, 1973; Burke et al., 1974).

This suggests a localized organization of motor control within masticatory muscles (van Eijden et al., 2001). In these studies, motor units were recruited during low levels of clenching, and were likely having originated from low-threshold and of slow-type motor units.

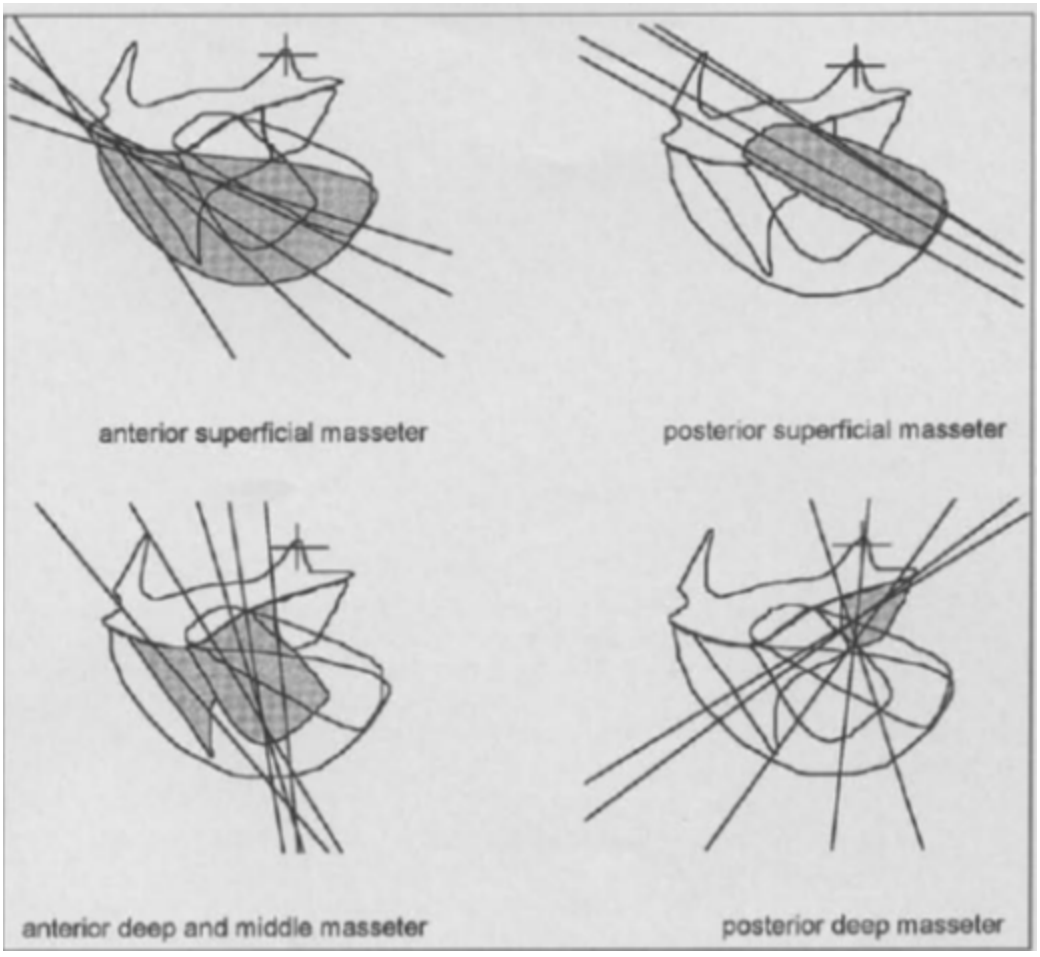


Figure I-6 Lateral view of left masseter of rabbit with projected lines of actions from different motor units (Turkawski et al 1998).

In 1998 Turkawski registered the magnitude, position, and direction of masseter motor unit forces after extracellular motoneuron stimulation in the rabbit's masseter muscle, and these studies confirmed the restriction of motor unit territories to a small sub-volume of the masseter muscle. Their results showed that the lines of action of motor units within the masseter muscle have indeed a large range of positions and directions, and that the variation of action lines was almost as large as the range of fiber directions inside the muscle (see Figure I-6) (van Eijden et al., 2001).

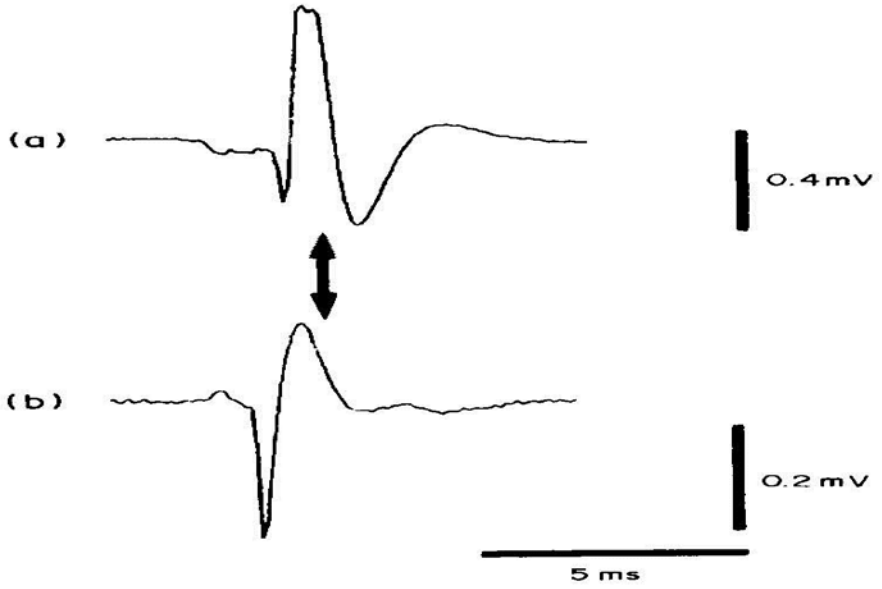


Figure I-7 Single motor unit, compound action potential recorded by triggering electrode "a" and by second electrode "b (by Hannam and McMillen 1991)

A sensitive method of stereotactically locating needle electrode recording sites within the human masseter muscle was developed by McMillan and Hannam in 1989 and this technique prompted them to map motor-unit territories in the masseter muscle using

multiple electrodes in known regions of the muscle. This technique provided a detailed understanding of motor-unit distribution and the functional compartmentalization of this complex muscle (the masseter) in particular, and other jaw elevator muscles in general. Figure I-7 Synchronous compound action potentials recorded by electrode “a” and “b” obtained from the unit investigated during an experimental session.

5.2 Activity of Motor Units during Voluntary Contraction

Motor unit activity in humans was detected with intramuscular electrodes, and their firing patterns received considerable attention following the endorsement by Tokizane and Shimazu (1964) that motor unit discharge in all muscles could be subdivided into two general categories: a 'tonic' pattern with low firing rates and regular discharge, and a 'kinetic' pattern with higher discharge rates and more irregular discharge patterns. Later studies generally failed to find a clear division of motor units into these groups, but suggested a more continuous range of firing properties between these extremes (Clamann.P 1970; Freund, H.J et al., 1975; Person R.S. et al., 1972; Stalberg et al., 1973).

The first recordings of single motor neuron activity were made by Sherrington's co-Nobel Laureate (1932), Edgar Adrian (1889– 1977) and American, Detlev Bronk (1897– 1975) who mechanically separated phrenic nerves into a few single or a small group of fibers in anesthetized rabbits using electrophysiological apparatus. In 1929 Adrian and Bronk, (1929) recorded the action potentials of the fibers of flexor and extensor muscles of the

cat hindlimb. In this study they used an audio phone which translated electrical activities into sound at the intensity proportional to the frequency of the impulses and also used a concentric needle electrode to record the compound action potential of muscle fibers innervated by a single motoneuron.

This revolutionary work on motor unit recording also showed that "… *the force exerted by a muscle during a voluntary contraction was the result of the concurrent recruitment of motor units and modulation of the rate at which they discharged action potentials"* (Duchateau et al., 2011). This work of Adrian and Bronk became a major step forward in motoneuron theory in that they built a bridge between motoneuron behavior during the voluntary contractions of human subjects and the mechanisms of motoneuron and reflex function in reduced animal preparations.

5.3 Recruitment and Firing Patterns

An extensive amount of work was performed during the 20th C which mainly focused on identify the directions that governed the activation of motor units during voluntary contractions. The recruitment order of motor units in a muscle, the factors influencing recruitment threshold force and recruitment order, the contribution of motor unit recruitment to muscle force, and the duration at which motor units can discharge action potential were the major issues which were studied (Adrian and Bronk, 1929; Mcphedran et al., 1965; Henneman et al., 1965; Stuart and Enoka, 1990).

5.3.1 Orderly Recruitment and Size Principle

The order of recruitment was questionable till the first half of the century and it was unclear whether the pattern of recruitment was stereotyped or whether it varied as a function of the task. After the initial studies of Adrian and Bronk (1929) that increases in force were achieved by the recruitment of additional motor units and an increase in discharge rate, Derek Denny-Brown (1901–1981) and Joseph Pennybacker (1907–1982) reported in 1938 that "a particular voluntary movement appears to begin always with discharge of the same motor unit. More powerful contraction is secured by the addition of more units". This fixed order of motor unit recruitment was called "orderly recruitment".

In 1957 Elwood Henneman demonstrated that whenever the intensity of electrical stimulation to the sciatic nerve was increased the single motor axons in a ventral root filament were recruited in order of the amplitude of their action potential. Furthermore, when the stimulus intensity was decreased the individual axons stopped discharging action potentials in the reverse order in which they were recruited. This finding, that was based on extracellular recordings and in which axon size was proportional to spike height, was generalized to conclude that the susceptibility to discharge is determined by motoneuron size, which had previously been shown to be proportional to axon size by Gasser in 1941. This observation was generalized to all neurons and it was hypothesized that the susceptibility of neurons to discharge varied as a function of their size.

In 1965, Henneman and his colleagues published an inspirational paper in the Journal of Neurophysiology which provided a detailed description of motor unit properties, motoneuron recruitment properties, and how the relations between these two sets of properties could be summarized in terms of a unifying principle that they called the “size principle”. The concept was later distinguished by Henneman (1977) as: “The amount of excitatory input required to discharge a motoneuron, the energy it transmits as impulses, the number of fibres it supplies, the contractile properties of the motor unit it innervates, its mean rate of firing and even its rate of protein synthesis are all closely correlated with its size. This set of experimental facts and interrelations has been called the ‘size principle’.”

The evaluation of size principle in human motor units can be credited to Stein and colleagues (Milner et al., 1973) who developed a technique of measuring the contractile properties of single motor units in the first dorsal interosseus muscle of the hand during voluntary isometric contractions (Stein et al., 1972; Milner et al., 1973). This technique utilizes the spike triggerred averaging method to describe the mechanical contribution of an identified single motor unit to the force exerted by the whole muscle (Mendell and Henneman, 1971). The data allowed them to identify the fact that human motor unit characteristics vary systematically with the threshold force for voluntary activation (Milner et al., 1973b). Based on these findings, they concluded that the recruitment of human motor units during contractions was in an orderly sequence and was dependent on motor unit force and these results were consistent with the size principle of Henneman (Henneman et al., 1965).

A number of different guides of motor unit size e.g. amplitude, conduction velocity of muscle fibers, amplitude of the evoked EMG waveform measured with intramuscular electrode have all confirmed the orderly recruitment of motor units during slow isometric contractions in a wide variety of human muscles (Calancie and Bawa,1990; Stuart and Enoka, 1990).

5.3.2 Recruitment Threshold

The motor unit pools consist of many low-threshold motor units and fewer high-threshold motor units (Thomas et al., 1986, Van et al., 1997), and the control of force during slow, low-force contractions relies mainly on the recruitment motor units (Enoka, 1995; Fuglevand et al., 1993; Heckman and Binder, 1991). The force that a muscle can exert depends upon the number of motor units activated and the rate at which the motoneurons discharge action potentials (Adrian and Bronk 1929). The force that a muscle exerts in its operating range, changes with the speed of the muscle contraction; the absolute force at which a motor unit is recruited decreases with an increase in contraction speed (Bigland and Lippold, 1954; Gydikov and Kosarov, 1974; Milner et al., 1973; Monster and Chan, 1977; Person and Kudina, 1972).

Similarly, the contribution of motor unit recruitment to muscle force can be explained by the seminal study of Jean-Edouard (1926–2009) and Godaux (1977a, 1977b) who recorded the spike-triggered average force (Stein et al., 1972) of motor units in the human tibialis anterior when the participants performed “ballistic” contractions. These studies

found that motor units that were activated earlier during ballistic contractions were approximately three times as likely to produce a given peak force during ballistic contractions compared with slow ramp contractions (Desmedt and Godaux, 1977a, 1977b).

These results suggested that most motor units are likely to be recruited while performing a rapid contraction and they found that the reduction in recruitment threshold with speed of contraction was related to the fiber-type composition of the muscle and was greater for units in slow-contracting muscles (e.g., soleus) compared with fast-contracting muscles (e.g., masseter) (Desmedt and Godaux, 1978, 1979). They concluded that: “The greater reduction in recruitment thresholds for slow muscles likely facilitates their ability to perform fast contractions and there were no differences in recruitment order between slow, ramp contractions and ballistic contractions” (Desmedt and Godaux, 1977a, 1977b). Later on it was suggested by some investigators that the peak force during rapid contractions might best be accomplished by the selective recruitment of fast motor units (Grimby and Hannerz, 1977; Tanji and Kato, 1973a).

5.3.3 Motor Units Discharge Rate

Studies on discharge rate of motor units were started in the early 1900s in animals (Adrian and Bronk 1928, Denny-Brown 1929, Sherrington 1930) and during volitional activity in humans (Adrian and Bronk, 1929). It was found by Adrian and Bronk (1929) and later on confirmed by Smith 1934 and Lindsley 1935 that a slight degree of voluntary

contraction sharply isolates action potentials at a rate which may begin as low as 6 a second and rises gradually as the contraction develops and in man that voluntary contraction is maintained like the reflex contractions in the cat by a series of nerve impulses which range from 5 to 50 or more a second in each nerve fibre.

In addition to this, Smith (1934) noted that there is great independence of rhythm during increases in muscle force in different units and Lindsley (1935) described that individual motor units usually began discharging action potentials at frequencies of 5 to 10 per second or even as slow as 3 per second, and could be increased to about 30 per second and even as high as 50 per second. Subsequently after the recognition that rate coding contributes significantly to the control of muscle force, investigators have studied the discharge patterns of motor units and the minimal and maximal limit of discharge rates.

In early 20th Century, it was found that the minimal discharge rate was due to an intrinsic mechanism located in the motoneuron itself (J.C. Eccles and H.E. Hoff 1932) and the first evidence of this mechanism was provided by Kernell in 1965 in which he distinguished that the lower limit for repetitive discharge by motoneurons in the cat was related to the duration of the after hyperpolarization (AHP) phase of the action potential. He found a strong correlation between the time course of the motoneuron AHP and the contractile kinetics of the corresponding muscle fibers. Further studies reported that humans are able to control discharge frequencies as low as 0.5 or 1 Hz and the observation that humans can discharge action potentials repetitively with interspike

intervals that were longer than the AHP duration (Eccles et al., 1958) directed Kudinaand Alexeeva (1992b) to conclude that AHP is not the only leading mechanism controlling the low firing rate of motoneurones under conditions of their natural activity in man.

Later on it was discovered by Hultborn that a motoneuron can produce self-sustained firing following a brief stimulus due to a calcium activated membrane potential (Hultborn et al., 1975, Hounsgaard et al, 1984). This gave emergence of a new era in the understanding of how discharge rates can be modulated. The recent work suggested that the motoneuron discharge rate and the conductance of AHP can be modulated by synaptic input scan in order to meet the needs of a particular task (Stauffer et al., 2007).

It was stated initially that the timing of the action potentials might have a significant influence on the force exerted by a motor unit (Gilson and Mills, 1941, Lindsley 1935, Seyffarth H. et al., 1940). The recording of motor unit activity during a fast contraction was first obtained from the flexor digitorum sublimis during a rapid flexion movement of the fingers (Gilson and Mills, 1941). These findings showed greater discharge rates during rapid movements than during sustained contractions and, for the first time, the existence of double discharges of action potentials by the same motor unit was observed. The concept of motor unit discharge during rapid contractions came when they compared the discharge of 24 units from the tibialis anterior muscle during both ramp and ballistic contractions and they found that the discharge rates ranged from 60 to 120 Hz during ballistic contractions.

The same discharge pattern was observed during ballistic contractions of the dorsal interosseus (Desmedt and Godaux, 1977b) and the masseter (Desmedt and Godaux, 1979), but these findings were observed during fast ramp contractions (Desmedt and Godaux, 1977). These authors also recognized that "Occasionally the spikes of the double response have been separated by as little as 10 milliseconds …" Subsequent studies reported similar high maximal rates (up to 100 Hz) during rapid, brief contractions (Grimby and Hannerz, 1977; Tanji and Kato, 1973b). Later on, double discharges by high threshold motor units during sustained isometric contractions was observed by Kudina and reported that the incidence varied across subjects (Kudina, 1974). These observations suggested that double discharges usually occur during tasks when force changes rather than during steady isometric contractions.

In contrast, an increase in the incidence of double discharges (ISI <30 ms) with an increase in contraction speed was found by Guarfinkel in 1972 (Gurfinkel et al., 1972). Motoneurons are considered to be more responsive to slight increases in synaptic input during the phase of delayed depolarization. The findings of Calvin and Schwindt, stated that second action potential in the double discharge is usually followed by a longer period of hyperpolarization (Calvin and Schwindt, 1972).

On the other hand, Bawa and Calancie (1983) proposed that the discharge pattern at the onset of ballistic contraction results from the motoneuron receiving massive amounts of synaptic input and not because of double discharges. More recently, Kudina and Alexeeva (1992a) suggested that a common mechanism, such as a "certain descending

synaptic input", is responsive for repetitive and single doublets during sustained, low-level contractions. Although the descending pathway is still unclear, these double discharges can be evoked by a magnetic stimulus applied over the motor cortex (Day et al., 1989; Bawa and Lemon., 1993). Moreover, the incidence of double discharges can be increased with training (Bawa and Calancie, 1983; Denslow, 1948; Van Cutsemet al., 1998); even though the role of double discharges in natural behavior is unknown (see Garland and Griffin, 1999).

6. HYPOTHESES EXPLAINING THE ASSOCIATION BETWEEN PAIN AND MOTOR ACTIVITY

Although jaw muscle pain and motor function are thought to be interrelated, the exact underlying mechanism of this relationship has remained a topic of research for many years (Murray and Peck 2007; Svensson P.,et al., 2001; van Dieën J., et al 2003; Stohler CS 1999; Mense et al., 2001; Graven-Nielsen et al., 2003). A number of theories have been proposed to explain the effect of pain on muscle activity; the two most frequently cited theories are the Vicious Cycle theory and the Pain Adaptation Model. It is important to explore the underlying mechanisms of this sensory-motor relationship as it is hoped that a better understanding will help to develop new management strategies for the treatment of pain, prevent persistence of symptoms and help to restore normal function. The principle findings from the literature are summarized below.

6.1 The Vicious Cycle Theory

In the early part of the 20th century, it was supposed that a common cause of pain was some abnormality in structure, movement patterns, posture, stress or other psychological disturbance that caused hyperactivity in a muscle which in turn, leads to spasm, fatigue, pain and dysfunction which causes more hyperactivity in the painful muscle and thus perpetuates the cycle (Travell and Simons, 1983; Johansson and Sojka, 1991; Mense 1993; Okeson 1996; Stohler 1999; Lund 2001; Mense et al., 2001). Despite the lack of good evidence, this idea became known as *The Vicious Cycle Theory* or *the pain-spasm-pain* theory of chronic pain and dysfunction (Travell, et al., 1942) (Figure 1-8). It is a simple theory that makes intuitive sense and was used to explain the aetiology of myofascial pain along with chronic lower back pain, fibromyalgia, tension-type headaches, and TMD. This theory is based on the debatable presence of hyperactivity in the muscles (Bodere et al., 2005).

The Vicious Cycle Theory hypothesizes that muscle activity increases in a stereotypical manner in pain, regardless of the task, and sustained activity leads to ischaemia and accumulation of algesic agents within the muscle and thus generating pain (Newham et al., 1994). A range of mechanisms have been proposed, and it was found that Ischemia alone is not the only factor which evokes muscle pain and mechanical factors (e.g., number of contractions, their duration and force) might also contribute to the development of strong muscle pain in humans (Mense S.1993; Newham et al., 1994).

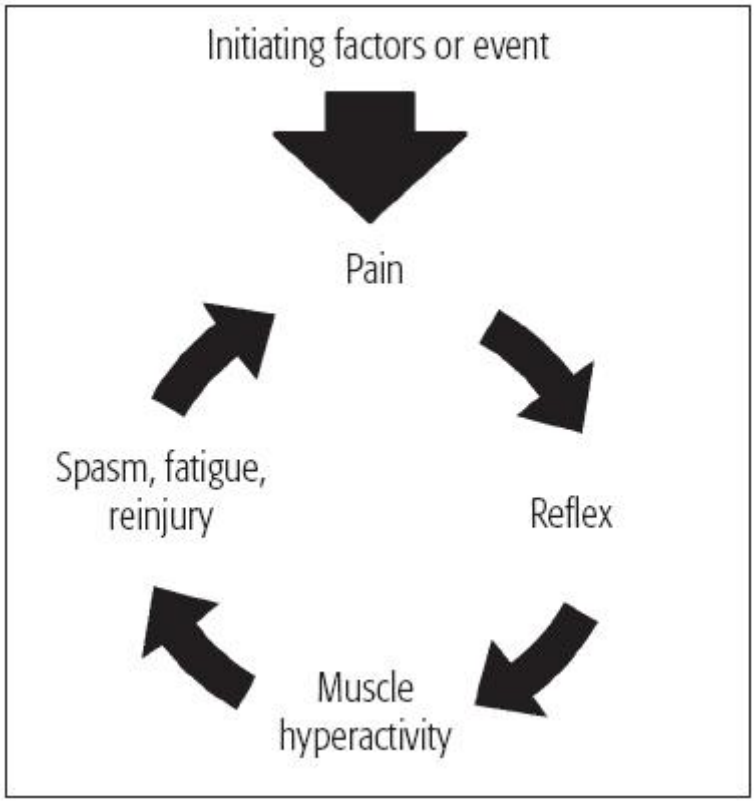


Figure I-8 The vicious cycle. This diagram shows the pain-spasm-pain cycle first described by Travel et al, based on the concept that activation of muscle nociceptors can lead to chronic pain because activation of nociceptors causes a tonic reflex contraction of all muscles.

Moreover, certain metabolic products e.g. lactate, potassium, or the lack of oxidation of metabolic products, hypoxia and the release of bradykinin (BK), prostaglandins (e.g., prostaglandin E2 [PGE2]), and calcitonin gene related peptide (CGRP), in association with reduced pH, may sensitize muscle nociceptors and lead to pain evoked by mechanical stimulation during contractions (Mense S.1993; Newham et al., 1994; Vecchiet et al., 1987).

Another mechanism of the neural basis for the vicious cycle theory proposes an increase in the sensitivity of muscle spindle afferents occurs due to inputs from group III and IV nociceptive afferents onto γ-motoneurons which control the sensitivity of the intrafusal muscle fibres within the muscle spindles (Johansson et al., 1991; for review, Hodges & Tucker, 2011). The stimulation of γ-motoneurones by nociceptive afferents increases the primary (Ia) and secondary (II) neural output of muscle spindles and thereby reflexively excites the α-motoneurones which cause further muscle contraction and thus sustains the cycle (for review see Knutson, 2000).

Although some experimental studies are in agreement with the Vicious Cycle Theory, most clinical and experimental studies are not supportive of this theory (Ashto et al., 1990; Hu et al., 1997; Cairns et al., 1998; for reviews, van Dieen et al. 2003; Murray and Peck 2007). Evidence in support of *the Vicious Cycle Theory* in humans is provided by some studies of neck and jaw musculature, where enhancement of the jaw stretch reflex was found with injections of glutamate into the right masseter and the right sternocleidomastoid muscle (Wang et al., 2004). Another study showed a positive correlation between VAS pain and myoelectrical stimulation of the sternocleodomastoid muscle across healthy young individuals (Ashton 1990).

The findings from some animal studies are also consistent With *the Vicious Cycle Theory*. One example of this is a study in which injections of analgesic chemicals (e.g. mustard oil) into the deep paraspinal tissues resulted in reversible sustained activation of the

masseter and neck muscles (Hu et al., 1993). Similarly other studies have shown an excitatory effect of mustard oil on the EMG activity of jaw muscles of the rat (Yu et al., 1994). It has also been illustrated in animal studies in jaw opening and closing muscles that the process of central sensitization is associated with increases in EMG activity resulting in an excessive movement and protection of articular and muscular tissue from further injury (Sessle B.J 2006). Based on the data obtained from experimental animals, muscle pain is believed to cause a reflex activation of fusimotor neurons and thereby increasing the sensitivity of muscle spindles to stretch and in this way initiate the cycle (Thunberg 2002).

Although this concept is still widely held by clinicians in particular, well-controlled studies have shown no statistically significant difference in resting EMG activity between painful and non-painful muscles or where changes have been shown, the changes are small in relation to control and both small increases or decreases have been noted (for reviews, van Dieen et al., 2003; Murray and Peck 2007; Matre et al., 1998, Mense S.1997 Graven-Nielsen et al., 2000; Bodéré, et al., 2005; Madeleine, 2005; Graven-Nielsen & Arendt-Nielsen, 2008; Peck et al., 2008; Sae-Lee, et al., 2008a; Xu et al., 2010). On the other hand, some studies have reported significant changes in jaw muscle activity during pain. One example of this is a comparison study of asymptomatic controls with myofascial and neuropathic pain patients with unilateral pain in which the pain patients showed significantly higher EMG activities at rest for both the temporal and masseter muscles bilaterally. These findings suggested a central origin for the muscle pain rather than muscle hyperactivity (Bodéré, et al., 2005).

Even though there is a lack of clinical evidence to support this theory, some clinical management strategies have been influenced by this theory and propose trying to break this cycle. One example of this is in the use of occlusal splints in the treatment of TMD. This is based on the concept that abnormalities in the dental occlusion are an important initiating factor that leads to muscle hyperactivity and TMD. However, there is very little evidence to support the view that an abnormality in the dental occlusion is a significant factor in the development of jaw muscle hyperactivity and thereby plays a role in the aetiology of TMD (Turp et al., 2008; Schindler et al., 2012). Similarly, experiments in jaw motor systems of animals have shown that stimulation of small-diameter jaw muscle (masseter muscle) or TMJ afferents usually inhibits masseter motoneurons and excites digastric motoneuron (Nakamura et al., 1973) and this finding is not consistent with *the Vicious Cycle Theory* which proposes an increased activity within a painful muscle.

It has been found that muscle spindle activity is, not facilitated by muscle nociception, but rather by a change in spindle sensitivity affecting the proprioceptive function (Masri et al., 2005; Graven-Neilson et al., 2008). However, the effects of muscle pain on the fusimotor system was explored by Fazalbhoy by recording afferent activity from foot and ankle extensor muscles whilst infusing hypertonic saline into the tibialis muscle of the ipsilateral leg, and showed no excitation of fusimotor neurons by group III and IV afferents. Taken together, these many studies allow a general conclusion that the vicious cycle theory appears to have no functional basis for the development of myalgia in human subjects (Fazalbhoy et al., 2013; Bent et al., 2013).

6.2 The Pain Adaptation Model

In 1991 Lund proposed a new model to explain the relationship between nociceptive afferent activity, a central pattern generator, motor function, and coordination of muscles. This model is known as *the Pain Adaptation Model* which includes an inhibitory and excitatory facilitation of motoneurons according to the functional phases (agonist or antagonist) of the painful muscle. In this way this model supports the need of assessing the functional effects of muscle pain in the various phases of dynamic contractions as well as in contractions without movements (static) and in resting conditions. This model proposes an increased muscle activity in the antagonistic phase and a decreased muscle activity in agonistic phase during muscle pain. The net effect of this is to decrease the force produced by the muscle and thereby lead to a decrease in movement velocity and amplitude during pain (Hodges & Tucker, 2011; Lund et al., 1991).

The Pain Adaptation Model redefined the role of pain in clinical settings, including persistent muscle pain conditions such as masticatory muscle pain, back pain and fibromyalgia. At the microneurological level, this model indicates how α-motoneurons are affected in situations of muscle pain in humans. The essential prerequisite for the model is a collection of premotor neurons, constituting the central pattern generator in the brainstem and groups of inhibitory and excitatory interneurons (Murray and Peck 2007 and 2008). Figure 1-9 shows a schematic presentation of pain adaptation model. Thin nociceptive afferents (dotted lines) from orofacial tissues influence both the central motor program and the central pattern generator (CPG) in the brainstem. There is a shift in the

normal drive to the alpha-motor neuron pool so that the jaw-closing muscles are less activated (dotted line) during pain in their agonist phase and more activated in their antagonist phase and same is true for the jaw-opening muscles. The consequences of this reorganization of the motor function are slower and smaller jaw movements.

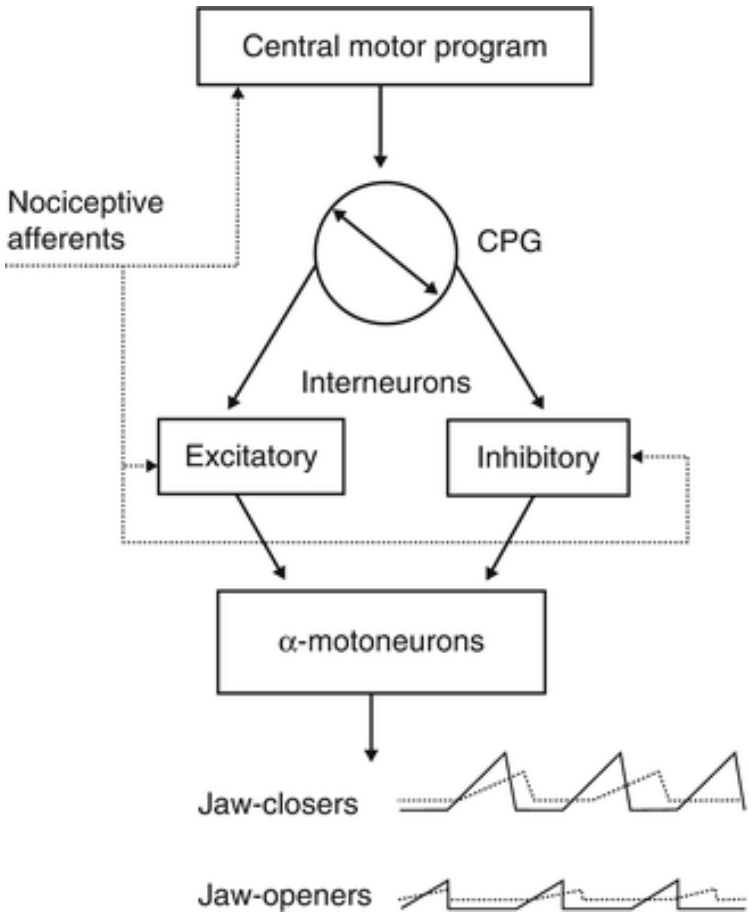


Figure I-9 The pain-adaptation model (Modified after Lund et al. 1991).

The pain-adaptation model explains many of the jaw-motor consequences of TMD pain, but does not provide any explanation for the origin of the pain. The effect of pain on α-motoneuron activity during resting muscle activity is generally reported to be small. The fact that some studies showing higher EMG activity at rest in humans were limited to

studies of the jaw muscles and the trunk muscles suggests that pain-related facial expressions linked to the action of the non-masticatory facial musculature could have contaminated the EMG recordings obtained from the muscles of mastication (Lund et al., 1991). In these studies, any increase in jaw muscle EMG activity could be due rather to facial muscle activation associated with the pain.

There are limited findings of increased EMG activity in humans during muscle pain compared to the animal studies (See in Crit Rev Sessle B.J 2000). In animal studies the most studies report clear responses in the jaw-opening muscles and weaker effects are noted in the jaw closing muscle (Sessle et al., 2000). This combined activation of openers and closers might be a reflex reaction in order to avoid movements.

As the motor unit firing rate is an important determinant of the force generated by a muscle, however, it is unclear how a constant force can be maintained in the presence of a pain-related decrease in motor unit firing rate. It has been stated that motor unit twitch properties might change as a compensatory mechanism for the decreased motor unit firing during pain, and increased twitch force of low-threshold motor units has been recorded during experimental muscle pain (Graven-Nielsen et al 2008). Recruitment of higher threshold motor units during pain is also a potential mechanism to explain the maintained force with reduced motor unit firing.

Reduced activity in both the agonistic and antagonistic muscles during muscle pain has been reported by Ervilha, without significantly impairing the movement amplitude or acceleration (Ervilha et al., 2004). In another study, reorganization of trapezius muscle activity during repetitive shoulder flexion has been found and this is manifest as decreased activity of the upper trapezius (where pain was induced), whereas the lower trapezius showed compensatory actions by increased muscle activity (Falla et al., 2007). In addition to this, recordings from the abdominal muscles have demonstrated a combination of an increase as well as decrease in its activity during experimental back pain (Hodges et al., 2006). Recent animal data also suggest a similar reorganized motor control pattern. The current experimental pain approach is essential when translating basic findings with clinical manifestations. The motor control assessment procedures can provide complementary clinical information and give further support for optimizing treatment regimens and developing prevention procedures for musculoskeletal pain (Graven Neilson et al., 2008).

Experimental pain studies in humans have also helped to clarify the relation between orofacial pain and jaw-motor function. It has been found that injections of hypertonic saline into the human masseter muscle causes a reduction of the agonist EMG activity during open-close jaw movements (empty and during gum-chewing), and a slowing down of the maximum velocity during jaw-opening and jaw-closing (Lund et al., 1991; Svensson et al., 1996). Hence, experimental muscle pain studies have often, but not always, shown a decrease in agonist EMG activity in the range of 10–15 %, a small increase in the antagonist EMG activity and modest reductions in maximum

displacements which is in accordance with other experimental pain studies and recording of muscle activity during gait and repetitive shoulder movements in humans (Graven-Nielsen et al., 2000 and 2008). Furthermore, experimental jaw muscle pain is associated with a short (<30–60 s) increase in EMG activity recorded with either intramuscular wire or surface EMG electrodes (Svensson et al., 2001). Normally, the magnitude of these increases in human EMG activity is comparatively small in the jaw-closing muscles and relatively short-lived, which does not seem to justify the term muscle hyperactivity. The findings of experimental pain studies from other parts of the body such as the trunk (Arendt-Nielsen et al., 1996), the lower limb (Graven-Nielsen et al., 1997; Farina et al., 2005) and the cervical region (Madeleine et al., 1999; Madeleine et al., 2006; Falla et al., 2007a) are also consistent with this model.

The experimental studies of animals have further supported the experimental findings of humans. For example, intramuscular injection of hypertonic saline in decerebrated rabbits causes a significant reduction in the agonist EMG activity, significant increases in the duration of the masticatory cycle and smaller amplitudes of jaw movement (Westberg et al., 1997). These data indicate that nociceptive activity has reflexly changed the dynamic jaw-motor function. Another study that has demonstrated facilitation of EMG activity during both jaw-opening and closing muscles following injection of various algesic substances into the deep craniofacial tissues of rats and cats, indicates that nociceptive afferents activate the excitatory pathways projecting to the alpha-motor neuron pools of the jaw-opening and jaw-closing muscles (Sessle B.J 2000). This co-contraction of the jaw-opening and jaw-closing muscles might serve as a “splinting” effect that reduces jaw

movements and in this sense, this finding is in accordance with the pain-adaptation model following injection of various algesic substances into the deep craniofacial tissues (Lund et al.,1991; Sessle B.J 2000).

However, several differences exist when comparing animal and human studies. One example of this is state of recording in which human pain studies are performed in conscious human beings in contrast to unconscious animals in which the influence of higher-order brain centres (during consciousness) cannot be excluded. Furthermore, many human studies are performed with a food bolus in the mouth, whereas animal studies have frequently involved empty open-close jaw movements. Thus the effect of pain might be influenced by motor programs related to such different types of jaw movements.

On the other hand, the findings of experimental and clinical studies are not always consistent with the basic idea of the Pain Adaptation Model that pain induces a uniform inhibition of agonist and facilitation of antagonist muscle activity with a resulting change in movement patterns (for review, see Murray & Peck, 2007). A recent study in the upper limb has shown a reduction in the discharge rates of low threshold motor units combined with recruitment of new units in response to hypertonic saline infusion into the infrapatellar fat pad and the flexor pollicis longus muscle. This alteration in motor unit recruitment pattern has been suggested as a likely reason for force maintenance despite the reduction in EMG activity in painful muscle (Tucker, et al., 2009). A similar study in masseter has investigated the effect of hypertonic saline induced muscle pain on single

motor unit recruitment pattern and revealed an increase, a decrease and no change in the single motor unit firing rate of the masseter muscle. In addition this study has shown a recruitment of additional units during pain (Minami et al., 2013).

Another study of human TMD patients has investigated the effects of pain from the TMJ and jaw muscles on masticatory motor activity and showed no significant differences of the root mean square EMG values of the anterior temporalis and masseter muscles in the painless condition in comparison to the painful condition when these muscles function as agonists. These findings are therefore not completely in line with the PAM (Stohler et al., 1988). Moreover, when these muscles function as antagonists, a significant difference was found in the painless condition compared to the painful condition, with greater root mean square EMG values detected during the painful condition. These latter findings are consistent with the Pain Adaptation Theory.

Nonetheless, the human experimental and animal studies are in general agreement with each other, and do provide some data sets that support the pain-adaptation model. However, there are many important issues that are needed to be explained. For example, the pain-adaptation model cannot explain how EMG activity is maintained in the presence of pain, as well as the origin of the pain. In addition, the possible long-term consequences of an adapted jaw-motor function to a chronic painful input are unknown. Therefore, both the Pain Adaptation Model and the Vicious Cycle Theory do not have universal support (reviewed Murray and Peck 2007; van Dieen et al., 2003).

6.3 The Integrated Pain Adaptation Model

As the literature has shown variations in the adaptation of motor control in response to pain, newer theories have been proposed to explain the association between pain and motor activity in more detail. One newer theory is the *Integrated Pain Adaptation Model* (IPAM) which has recently been proposed by Murray and Peck in 2007. In fact, the IPAM is not a separate entity from the pain adaptation model but is considered to be an update of the model in view of the most recent data with a renewed focus on the importance of the brain in regulating the unique motor responses.

The Integrated Pain Adaptation Model has addressed the individual motor response of pain in relation to the sensory-discriminative (e.g. location, intensity, duration of pain), cognitive-evaluative (beliefs based on previous experiences), as well as motivational-affective (e.g. an unpleasant emotional experience etc.) dimensions of pain and has attempted to provide an explanation regarding the effects of psychological factors on the pain-motor interaction (Murray & Peck, 2007). For example, the effect of pain on motor unit activity may result in a new recruitment strategy (altered/reorganized activity) in a unique response with the aim of minimizing pain and maintaining homeostasis (Murray & Peck, 2007). There is evidence of inter-individual variability in the behavioral responses to pain, (Mogil JS.1999, Raber and Devor M.2002) with both genetic and psychosocial variables playing fundamental roles. In another study, it has been found that the relationship between the psychological constructs and pain perception differs markedly between men and women (Michel A.et al., 2013).

According to this model, in the entire sensorimotor system, a complex change in activity occurs which is influenced by individual responses (both sensory and psychological) to pain and the anatomical and functional complexity of the sensorimotor system (Figure I-10). The change in activity of muscle occurs irrespective of the role it plays during function (i.e. whether the muscle is an agonist or an antagonist) and these changes sometimes might lead to further pain or injury (Murray & Peck, 2007).

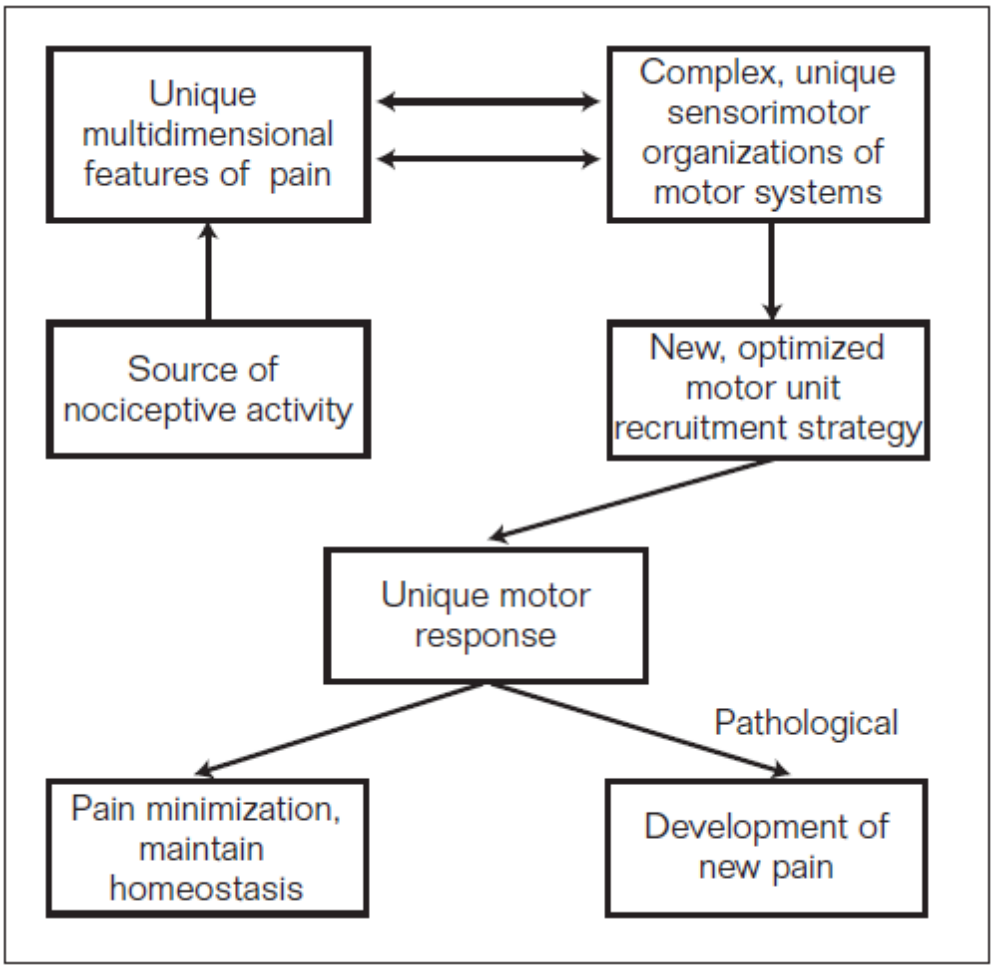


Figure I-10 Outlining the essential components of the integrated pain adaptation model (From Murray and Peck 2007).

A comprehensive review on lower back pain patients done by Van Dieen et al showed the effects of back pain on trunk muscle activity were not consistent with either of the previous two models of pain and proposed that these changes were task dependent and were related to individual behavioural response (Van Dieen et al., 2003). These earlier findings in the spinal motor system lend support to this newer model.

Some recent examples of a data consistent with the proposals of the IPAM are the studies of orofacial tissues in humans and experimental animals which has shown a reduction in the excitability of the primary motor cortex in experimental pain (Boudreau et al., 2007; Adachi et al., 2008; Nash et al., 2010). These findings demonstrate that pain is having effects not just at the spinal/brainstem level as proposed by the earlier models, but that higher centres of the brain appear also to be involved. Another example is a study showing a correlation between psychological variables and kinematic variables between female myofascial temporomandibular disorder (TMD) patients and an age matched control females (Brandini et al., 2011). This study has shown significant positive correlations between depression and jaw amplitude and stress and jaw velocity (Brandini, 2011). These set of studies point to an important role for higher motor centres and psychological variables in influencing the interaction between pain and motor activity.

6.4 Fear Avoidance Model

There is good evidence that psychological factors play an essential role in the transition from acute to chronic pain and disability (Turner et al., 2001; Lobbezoo et al., 2002; Fillingim, 2005; Murray and Peck, 2007; Aggarwal et al., 2010; Hall et al., 2011). Another recent model known as the fear-avoidance model (Leeuw et al., 2007) explains the influence of psychological factors in the transition from acute to chronic pain.

Pain catastrophizing is the process whereby an individual interprets pain as being extremely threatening and it has been observed that high-catastrophizing individuals have a tendency to focus excessively on a pain sensation (rumination) and to experience a sense of helplessness (Leeuw et al., 2007). This model proposes that high-catastrophizing individuals develop avoidance behaviors e.g. develop new movement and muscle activity in order to avoid pain. This adoptive behavior can be useful in the short term (e.g. in terms of reduction of pain) but in the long term can lead to disuse, disability, depression, and further pain. Low-catastrophizing individuals, on the other hand, do not develop an unrealistic fear of movement, and antagonize their pain, which promotes functional recovery (Sessle B.J 2014).

6.5 New Theory of Motor Adaptation to Pain (Tucker and Hodges)

Another theory has recently been proposed by Tucker and Hodges in 2011 which explains the motor adaptation to pain on the basis that the adaptation to pain aims to

reduce the pain and protect the painful part in more flexible manner than as provided by VCT and PAM based on the existing data at the micro (single motoneurone) and macro (whole muscle behavior) levels. Five fundamental key elements of this theory (Hodges and Tucker, 2011) are:

1. The adaptation to pain involves redistribution of activity within and between muscles instead of just excitation or inhibition of muscles.

The first element of this theory provides an explanation for how force is maintained despite a reduced motoneurone discharge rate during pain (Farina D, Arendt-Nielsen L 2005, Hodges PW 2008) by showing recruitment of new motor units during pain. In this way, this newer model explains the maintenance of force during pain which could not be readily explained by the previous theories (Graven-Nielsen et al., 2000; Tucker et al., 2009, Tucker and Hodges, 2009).

Figure 1-11 shows an example of this redistribution of activity in flexor pollicis longus muscle (FPL) during recording of single motor unit activity. 3 SMUs were recruited during both no-pain and pain trials (A, C, E) but which showed a decrease in discharge rate during pain. Unit D was de-recruited during pain. 3 new SMUs (B, F, and G) which were not active in the no-pain conditions were recruited during pain. These features indicate a change in the population of active units during pain in order to maintain force output. A recent of masseter also found similar results and has shown redistribution of activity in masseter during recording of single motor units (Minami et al 2013).

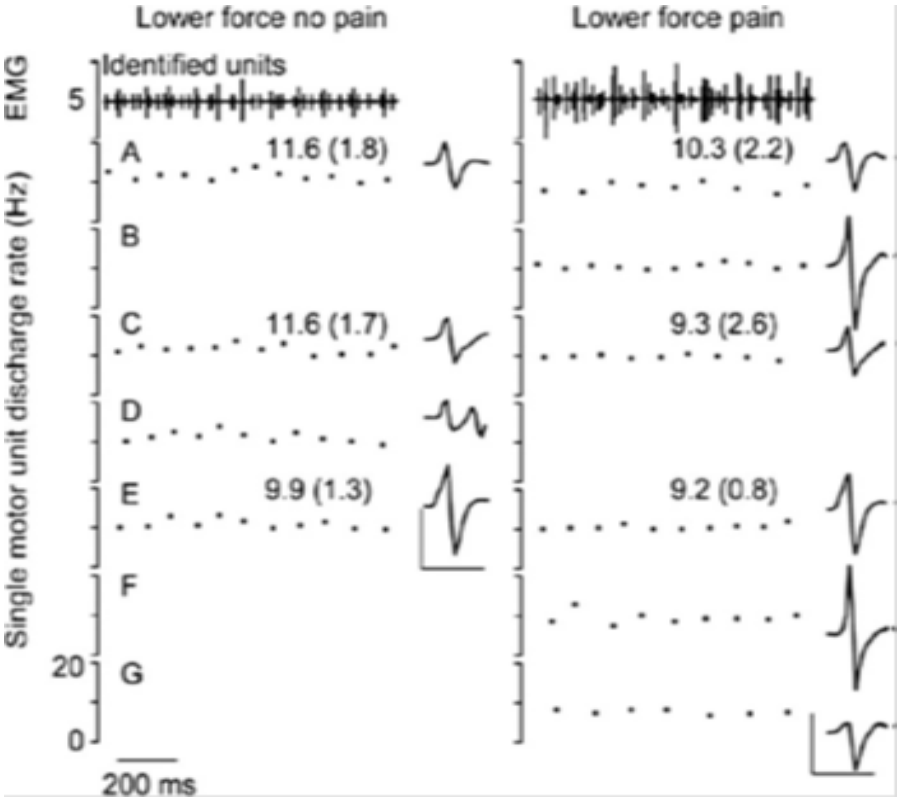


Figure I-11 Redistribution of activity within a muscle adapted from (Tucker et al 2009) SMUs (A, C, E) were recruited during both no-pain and pain trials. Unit D becomes inactive during pain and 3 SMUs (B, F, and G) were not active in the no-pain conditions and become active during pain.

2. Another key element of this theory is that adaptation to pain changes the mechanical behaviour and examples of these changes are modified movement and stiffness.

Recent studies of effects of pain on jaw movement emphasize that adaptation is common and the nature of the change can vary between individuals (Peck et al., 2007; Sae-Lee D et al., 2008). Normally, the brain activates motor units in accordance to the

needs of producing appropriate movement but in pain, it may cause the individual organization of the sensorimotor system to react in a unique way to the pain (Murray and Peck, 2007). Because of multidimensional nature of pain this interaction between pain and the sensorimotor system is unique between individuals (Sessle BJ.2000., Mogil JS.1999. Casey KL.1999).

3. Adaptation to pain leads to protection from further pain or injury following from these motor changes.

This element of the model is also consistent with the hypothetical model of Murray and Peck that the nervous system may search for a movement pattern that is less painful during painful jaw movements (Murray and Peck, 2007). The presence of adaptation to pain has been exemplified by the hypertonic saline injections into the infrapatellar fatpad and which resulted in a redistribution of activity in vasti muscle. It was suggested that the redistributed muscle activity changed the load and reduced the mechanical irritation of this structure (Tucker KJ, Hodges PW.2010). In this theory Tucker and Hodges state that the nervous system has several options to meet the goal of protection of the skeletomotor system. In the jaw motor system, this may arise through reduced activity of masseter (Sevenson et al., 1997) or reduced jaw movement amplitude (Akhter et al., 2014) during painful jaw movement. In neck pain, there may be an increased activity of the sternocleodomastoid muscle in neck pain (Johnston et al., 2008), or a combination of both increases and a decreases in abdominal movements during experimental muscle pain (Hodges et al, 2006, Tucker

and Hodges, 2011; Minami et al 2013). Whether or not all of these effects come about to achieve the goal of protection of the skeletomotor system remain to be determined.

The following figure has summarized key elements of this theory.

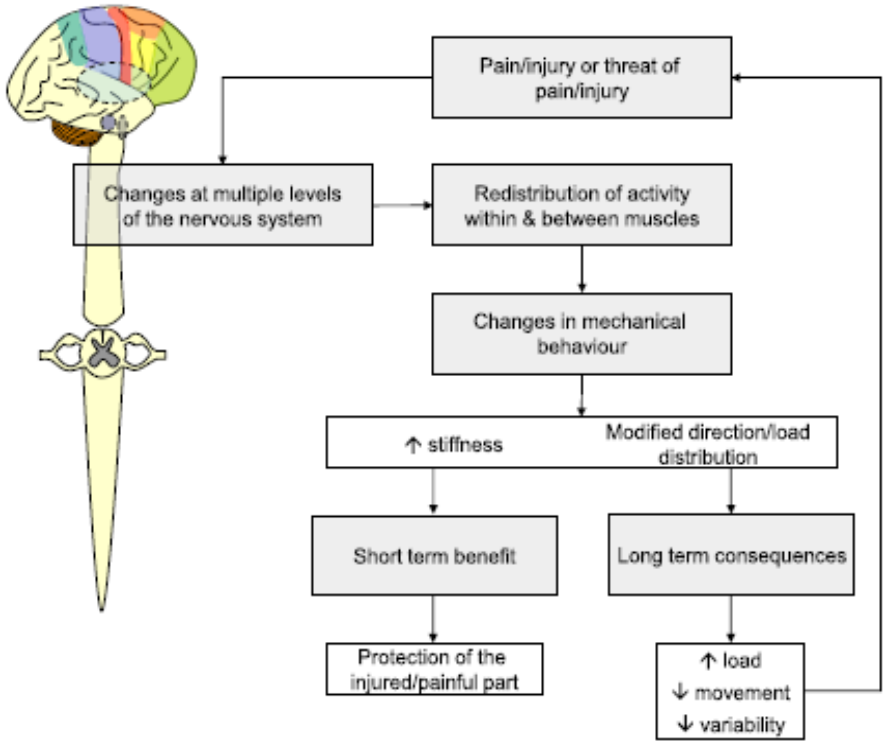


Fig. 3. New theory of motor adaptation to pain.

Figure I-12 New Theory of motor adaptation to pain (Hodges and Tucker 2011)

4. Adaptation to pain involves complementary, additive, or competitive changes at multiple levels of the motor system instead of a simple change in excitability.

An explanation of this could be the increased excitability of motoneurone. While studying the effect of group III and IV afferents on motor cortex, Martin found increased motoneurone excitability accompanied with decreased cortical excitability. Similarly Schabrun, found increased motoneurone excitability with intracortical inhibition and these changes had two different mechanisms (Martin et al., 2008, Schabrun and Hodges, 2010). Adachi studied the effects of noxious lingual stimulation on excitability of face primary motor cortex (MI) and observed inhibitory effects on prolonged neuroplastic changes manifested as a decrease neuroplastic changes manifested as a decrease in face MI excitability (Adachi et al., 2008 ; Nash et al., 2010).

5. Adaptation to pain has a short-term benefit but has the potential for long-term consequences due to factors such as increased load, decreased movement, and decreased variability.

These proposed negative outcomes of adaptations are not likely to be immediate but require a period of maintenance or repetition to influence tissue health. The ability of the nervous system to adapt ultimately determines the capacity to minimize any potential negative impact. Variability is a fundamental property of biological systems, and its role in motor learning and control is central to the study of movement and posture. A consistent task performance seems to require variability in its motor constituents, which enables adaptation to altered task demands without compromising performance. One example of this is in the findings of Lorimer and Moseley in a back pain study which

showed that pain induced variability in normal posture and it is unlikely to restore muscle recruitment patterns to a pre-pain state (Moseley G.L. et al., 2006).

7. PSYCHOLOGICAL VARIABLES AND ASSOCIATIONS WITH MOTOR ACTIVITY IN PAIN

"There has been a growing recognition that pain is a complex perceptual experience influenced by a wide range of psychosocial factors, including emotions, social and environmental context, sociocultural background, the meaning of pain to the person, beliefs, attitudes, expectations, as well as biological factors. Chronic pain if persist for months and years influence all aspects of a person's functioning including emotional, social and occupational. *A successfully treating chronic pain patients requires attention not only to the organic basis of the symptoms but also to the range of factors that modulate nociception and moderate the pain experience and related disability*" (Turk & Okifuji, 2002; Aggarwal et al., 2010).

In the presence of pain, psychological factors have been found to be significantly correlated with measures of motor function in both trigeminal and spinal systems. Some psychological factors that have been studied are anxiety, stress and/or fear of movement, depression and catastrophizing (Lobbezoo et al., 2002; Leeuw et al., 2007; Alschuler et al., 2008; Brandini et al., 2011; Zhao et al., 2012; Akhter et al., 2014). Recently, it has been found that the interaction between masseter muscle pain and jaw muscle and brain activity are associated with psychological variables, and catastrophic thinking shows a

significant association with jaw movement as well as activity in motor and sensory integrative regions (Akhter et al., 2014; Henderson LA.et al., 2015).

A number of studies have examined the role of psychosocial stressors, parafunctions and other psychological and behavioral processes in TMD pain and have reported a positive relationship between stress and TMD in children, adolescents and adults (Korszun et al., 1996 Dworkin et al., 1990). Other studies have found that psychological disturbances are not uniformly distributed among patients with TMD and are more common in patients with chronic muscular pain than those with non-painful disk and joint problems (Hudson et al., 1992; Crofford et al., 1996 Korszun et al., 1998; Schmidt et al., 2009). While exploring psychological parameters, it has been observed that pain patients experience more anxiety than appropriate control participants and it has also suggested that these patients also feel less in control of their own outcomes than normal participants (Carlson et al., 1998).

Psychological factors have also found to be important risk factors for the development of TMD and it has been observed that, during pain, the strategy to carry out mastication may differ between individuals, depending on psychological status or emotions (Cram et al., 1988). Therefore, in order to clarify the possible associations between motor function and psychological status, it is necessary to analyze both physical parameters such as force and EMG activity and psychological conditions with the use of questionnaires such as the Jaw

Functional Limitation Scale (JFLS) and the Pain Catastrophizing Scale (PCS), Depression anxiety and stress scale (DASS) (Sulliven et al., 1995; Lovibond et al., 1996).

The predictive value of psychological variables in lower back pain has been investigated and these variables have been found to be important in the maintenance of chronic lower back pain. For example, the extreme tendency to avoid physical activities in some patients can lead to a decrease in physical fitness and can contribute to a long-term deconditioning and thereby lead to further back pain under normal physical strains (Hasenbring et al 1994). Based on the preceding evidence and the biopsychosocial model of TMD proposed by Dworkin et al, a heuristic model of causal influences contributing to the onset and persistence of TMD and related conditions has been created. This model proposes that TMD, and its associated signs and symptoms, are influenced most proximally by psychological distress and pain amplification (Dworkin et al., 1992; Maixner et al., 2011; Akhter et al., 2014).

When pain is moderate to severe, it impairs function and the fact that it is refractory to treatment is associated with depressive symptoms. Pain also causes impairment in social function and work abilities as well as functional limitations that lower the quality of life, and increase health care utilization (Schmidt et al., 2009; Michel et al., 2013; for review Sessle B.J 2012). Moreover, a literature review by Mathew has established the relationship between depression and pain and has found that depression complicates pain

complaints such as greater intensity and longer duration. Depression also complicates the management of pain and is associated with poor pain outcome (Matthew et al., 2003).

Patients with altered psychological state (e.g. depression) often present with a complex set of overlapping symptoms, including emotional and unexplained physical complaints such as pain, fatigue, sleep disturbances etc. (Korszun et al., 1998; Glaros et al., 2005; Otis JD, et al., 2003; Thibodeau et al., 2013). Various studies have shown that brain regions involved in the generation of emotion such as medial prefrontal, insular and anterior temporal cortices, hypothalamus and amygdala send projections to brainstem structures which are involved in pain modulation (Fields H et al., 2000). The underlying mechanisms of the relationship between psychological variables and sensory and motor system are not fully understood but there are data that suggest that high-catastrophizing individuals have enhanced central sensitization processes in nociceptive pathways within the central nervous system (Quartana et al., 2009). There is a need of future studies in chronic orofacial pain groups with the view of developing new interventions focusing on psychological variables in relation to motor activity.

8. ELECTROMYOGRPAHY AND STANDARDIZED JAW TASKS

Surface electromyography is a method used to analyse the activity of muscle during rest or functional activities. This technique has been used widely to describe the role of a muscle in specific movements. The EMG signal in the masseter muscle is the summation of the action potentials of many muscle fibers from many motor units.

Several factors can influence the recording of the EMG signal and these can be divided into physiological factors, for example type of muscle fibers, nerve fiber conduction, body temperature; anatomical factors, for example diameter of the muscle fiber, position of the muscle in relation to the electrode and thickness of the skin; and technical factors, related to instrumentation and involving aspects related to the capture and processing of data (Fridlund et al., 1995,).

Three main types of electrodes are used for recording EMG activity from muscles and these are; isolated surface electrodes, multiple surface electrodes, and implanted electrodes. Isolated surface electrodes record the activity from part of an isolated muscle; multiple surface electrodes record the activity of a larger region of a muscle or from several muscle groups; implanted electrodes are intramuscular electrodes that are used when muscles are not accessible to surface electrodes. Despite the limitations inherent in EMG recordings, the electromyogram has proven to be a valuable methodology in both spinal and trigeminal motor systems by providing significant information about muscle activity during different movements. In the trigeminal motor system, the recording of jaw movements or jaw forces at the same time as recording jaw muscle EMG activity has significantly improved the interpretation of the EMG as activity in a muscle may not always necessarily imply a certain defined movement for example but can be associated with different jaw movements (Markopolous, 2010).

8.1 Bite Force Recording

Recording of bite force is a useful mean of evaluating jaw muscle activity and function. Bite-force performance has been used by many studies to evaluate the predictions made by computer-based biomechanical models (Curtis et al., 2010a; Gröning et al., 2013). It is

an indicator of the functional state of the masticatory system that results from the combined action of the jaw elevator muscles modified by craniomandibular biomechanics, their muscle cross sections, muscle sarcomere length and reflex mechanisms (Hagberg et al., 1987). The reflex mechanisms responsible for controlling the bite force can be activated by sensory receptors such as periodontal and intra-dental mechanoreceptors, receptors in the temporomandibular joint and/or muscles spindles of jaw muscles (Oki et al., 2003; Yamamura et al., 2008).

It can also be observed that the maximum bite force progressively increases from the incisor group to the first molar group in both genders and bite surfaces (Review Duygu Koc 2010; Calderon Pdos et al., 2006). Individual bite force determination has been widely used to understand the mechanics of mastication for studies on the biomechanics of prosthetic devices and it has been considered important in the diagnosis of disturbances of the stomatognathic system (Fernandes et al., 2003; Calderon et al., 2006).

Bite force measurements can be made directly or indirectly. The direct method of measuring bite force involves the use of a suitable transducer placed between one or more pairs of upper and lower teeth. The indirect method of evaluation of the bite force is through studies of the associated EMG activity recorded from the elevator muscles of the mandible and the data obtained gives information about the bite force. Several studies have showed a linear relationship between jaw muscle EMG activity potentials and direct bite force measurements, especially at a submaximal level (Ferrario et al., 2004).

According to the literature, the recorded values of maximum bite force vary widely among individuals (Koc, et al., 2010; Alhaijaet al., 2010) and this great variation in bite force values depends on many factors which can be divided into person-related and technical-related factors. Individual factors are related to biomechanical, physiological and anatomical craniofacial variables such as age, gender, bite position, unilateral or bilateral biting, facial morphology, signs and symptoms of temporomandibular disorders, dental status, and functional disturbances of the masticatory system and head posture at the time of measurement (Hagberg et al., 1987; Manns et al., 1979). In addition to these biological factors, technical factors depend on the measuring method, use of different recording devices and the mechanical characteristics of the bite force recording systems such as position of recording devices in the dental arch unilateral or bilateral measurements, and use of acrylic splints (Oki et al., 2003; Yamamura et al .,2008).

Another important factor is the position of the transducer in the dental arch which may influence the different muscles involved in the force production. It has been observed that if the transducer is placed anteriorly between the incisor teeth, with a resultant mandibular protrusion, the masseter muscle will produce most of the force together with the medial pterygoid muscle. Conversely, posterior placement of the transducer is associated with activity in the anterior fibers of temporalis which makes a greater contribution to the effort (Tortopidis et al., 1998; Koc, et al., 2010). The use of acrylic splints provides a comfortable surface for maximum bite force (Fernandes et al., 2003) and provides a standard position for the transducer for each individual and for each recording session (Fernandes et al., 2003; Tortopidis et al., 1998). Moreover, it has been

found that the involvement of recording site can also influence the measurement of bite force and Shinogaya et al have compared bilateral and unilateral bite force measurements with different transducers and reported that the activity of the masseter muscle increased by about 50% during bilateral clenching compared to unilateral clenching (Shinogaya et al., 2000).

The literature has provided evidence that primary motor cortex play an important role in generation of bite force, movement and displacement (Ashe et al., 1997' Fetz et al., 1994; Humphrey et al., 1970). There is a recent evidence of inhibitory effects of pain on MI activity (Farina et al., 2001; Le Pera et al., 2001; Valeriani et al., 1999, 2001; Nash et al., 2010; Adachi et al., 2008). Adachi et al (2008) showed that intraoral noxious stimulation resulted in prolonged neuroplastic changes which manifested as a decrease in the excitability the face MI. These effects appeared to occur in those parts of the face MI that provided motor output to the orofacial region receiving the noxious stimulation. These findings provide data consistent with the notion of a reorganization of motor activity within the jaw motor system in pain. These inhibitory effects on face MI activity may therefore require a reorganization of activity within the face MI in order to allow a voluntary jaw motor task to be completed.

9. GROSS ANATOMY OF MASSETER MUSCLE AND ITS FUNCTIONS

The human masseter muscle has a complex morphological structure consisting of several different portions with different fiber directions and sarcomere lengths (Ebert, 1938/39; Schumacher, 1961). The internal complexity of the masseter has been described in rat (Nordstrom and Yemm, 1974), pig (Herring et al., 1979), rabbit (Weijs and van der Wielen-Drent, 1983) and human (van Eijden and Raadsheer, 1992; van Eijden et al., 1997). The muscle has been divided mainly into superficial and deep parts (Belser and Hannam, 1986; Wood, 1987) and has been demonstrated to be functionally heterogeneous (Blanksma et al., 1992; McMillan and Hannam, 1992; McMillan, 1993; Blanksma and Van Eijden, 1995; Goto et al., 2001; Schindler et al., 2005). Functional heterogeneity refers to the ability for differential activation of sub-compartments of the muscle according to the biomechanical needs of the task (Palla and Farella, 2010). Heterogeneity has also been observed in the muscle's internal architectural design (Munro and Griffin, 1970; Vitti and Basmojian, 1977; Van Eijden, 1990).

In order to refine the understanding of the multilayered internal architecture of the masseter muscle, recently Widmer has studied the development of the complex organization of the masseter and has found that the complexity of the masseter muscle architecture is established prior to birth (See Figure 1-15) (Widmer et al., 2007).

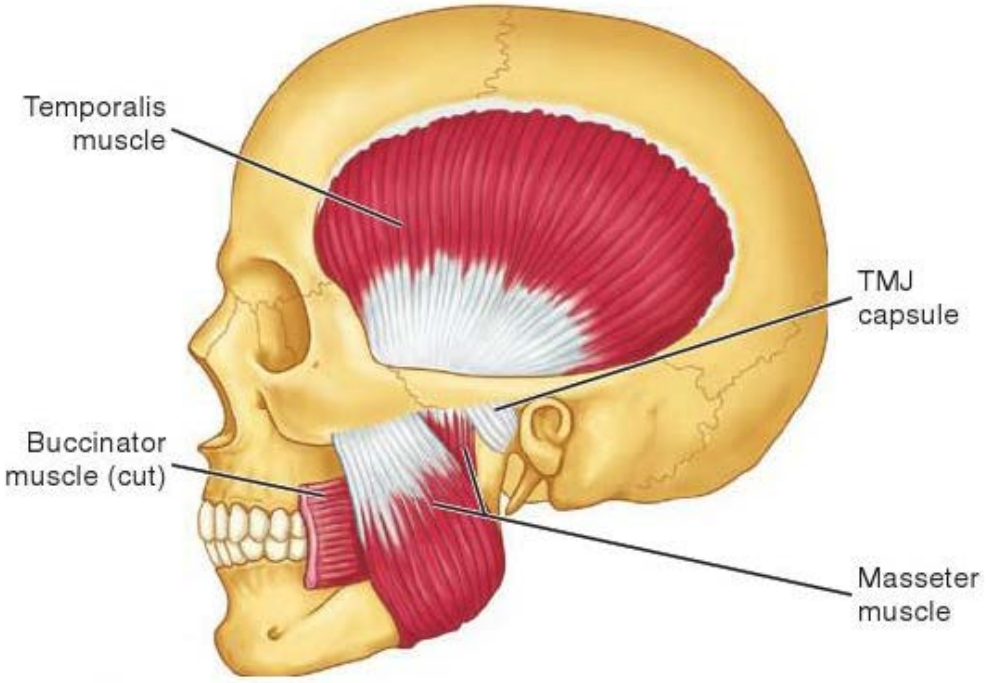


Figure I-13 shows masticatory muscles including masseter and temporalis.

Initially, all jaw muscles are derived from segmentation of a single muscle mass at the 11th day of gestation, and partitioning of this single mass into specific jaw muscles and compartments is associated with the branching of the trigeminal nerve (Melzer et al., 2000).

The masseter muscle takes its origin from the zygomatic arch and extends to the ramus and body of the mandible. Its insertion is large and extends from the region of the second molar at the lateral surface of the mandible to the posterior lateral surface of the ramus and in some individuals there is some insertion onto the orbicularis oris muscle (one of the muscle of facial expressions). The masseter is covered partly and variably by parotid gland tissue and its superficial part is separated from a deeper layer of muscle only at the

posterior upper part of the muscle (Nelson 2009). The superficial head (the larger part) of the masseter arises by a thick aponeurosis from the maxillary process of the zygomatic bone, and from the anterior two-thirds of the inferior border of the zygomatic arch and is inserted into the angle of the mandible and inferior half of the lateral surface of the ramus of the mandible (See Table 1-2) (Weaker, 2013).

The deep head (smaller part) is a more muscular in texture and it arises from the posterior third of the lower border and medial surface of the zygomatic arch and is inserted into the upper half of the ramus as high as the coronoid process of the mandible (see Figure 1-13). Given this anatomical arrangement, it may be an antagonist to the posterior temporalis and synergistic for the lateral pterygoid muscle (Nelson 2009). The masseter has been divided into eight subvolumes (See Figure 1-14), its aponeurosis exhibit a fan shape arrangement in both anteroposterior and mediolateral directions and its content vary systematically across different muscle sub volumes, being denser in the anterior-cranial portions close to the zygomatic arch and in the deep masseter subvolumes. The aponeurosis act as mechanical buffers to protect muscle fibres from sudden high-strain injury and plays an additional role in storing and releasing energy during motion (Griffiths, 1991; Gaudy et al., 2000; Cioff et al., 2011).

Table I-2 An Overview of the Masseter Muscle showing origin, insertion, main actions, nerve supply, and some additional information for the superficial and the deep head (Modified from Norton, 2012).

Muscle	Origin	Insertion	Main Actions	Nerve supply	Additional information
Masseter : Superficial head (larger part)	Inferior border of the 2/3 of the zygomatic arch	Angle of mandible Inferior and lateral parts of the mandibular ramus	Elevates mandible Protrudes mandible (superficial head) Aids in lateral excursion of the mandible	Masseteric branch from the mandibular division of the trigeminal nerve	Superficial head's fibres run posteroinferiorly The parotid duct, transverse facial artery, and branches of the facial nerve pass superficial to the masseter muscle.
Masseter : Deep head (smaller part)		Medial border of the zygomatic arch Inferior border of the posterior 1/3 of the zygomatic arch		Superolateral mandibular ramus Coronoid process	

The histochemical and morphological fiber characteristics of the masseter muscle were first studied by Eriksson and Thornell (1983) and they divided the masseter muscle into five parts. The masseter muscle has a complex morphological structure, mainly composed of three layers: superficial, intermediate and deep (Schumacher et al., 1961) and predominantly consists of type I fibers except for its posterior superficial portion. Type I fibers have high oxidative enzyme activity which shows its high resistance to fatigue (Eriksson and Thornell, 1983). The posterior part of the superficial masseter mainly

contains type IIB fibers that belong to fast twitch and rapidly contracting motor units which are sensitive to fatigue. This indicates a capacity in the molar region for high muscular tension. Ringqvist (1974) described a positive correlation between type II fiber diameter of the masseter muscle and bite force.

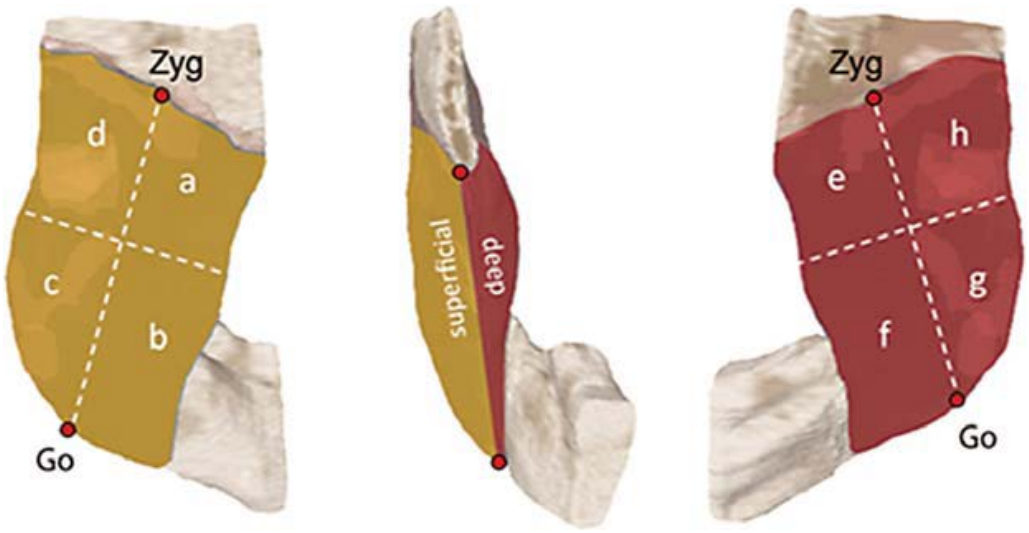


Figure I-14 shows Identification of eight sub-volumes of masseter muscle. The sagittal plane, perpendicular to the other reference planes, and passing through zygomatic and gonion points, was considered as the sagittal reference plane.

Human masticatory muscles express a large variation in phenotype, especially in their myosin heavy chain (MHC) composition (Sciote,et al., 1994; Morris T.J et al., 2001), both between individuals and between different portions of the muscle (Vignon et al., 1980; Gwénaël et al., 2011). Muscle fiber phenotypes can be sufficiently assessed by

ATPase staining and it has been found that the masseter contains additional (developmental) and atrial (α-cardiac) myosin isoforms which can be characterized by immunostaining in comparison to the limb muscle, which contains only the 3 commonest MHC isoforms [I (slow), IIA (fast), IIX (fastest)] (Bredman et al., 1992). Recently histochemical and immunohistochemical studies (Wolff et al., 1986; Fitts et al., 2001;Raoul,G 2006; Mounier et al., 2009; Sciote,J.J, et al., 2012) have pointed to differences in fiber type composition across muscles and muscle portions and found that the relative proportion of type II fibers is larger in the superficial than in the deep masseter.

Furthermore, at lower bite force levels, the deep masseter (with less type II fibers and usually further away from the registering EMG electrodes) is preferentially activated (Schindler HJ et al., 2014; Terebesi et al., 2015), and at higher levels relatively more activity is likely to occur in the superficial masseter (with more type II fibers and closer to the electrodes) is added (Farella et al., 2002). Recently, intermediate (IM) fibers have been described histochemically and it has been observed that the frequency of IM fibers in the masseter was higher in patients with poor occlusion than in those with good occlusion (Sciote et al., 2013, critical review). It has been observed that motor units in anterior masseter showed more variability in force production than in posterior part (Van Ejiden 1998).

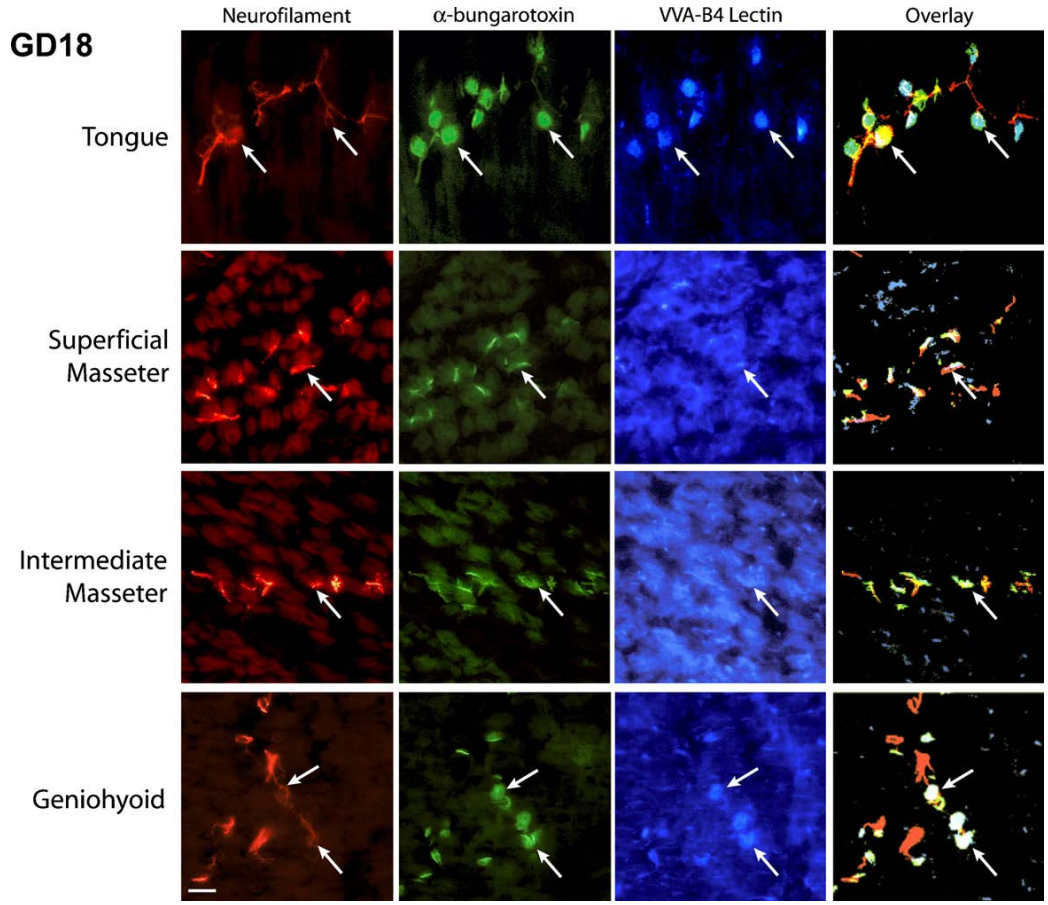


Figure I-15 The neuromuscular endplate development in the masseter is delayed compared with development in the tongue and geniohyoid muscles (by C.G. Widmer; 2007).

The masseter is a powerful elevator muscle which may assist in the protrusion of mandible. The centre of the lower third of the masseter is about 2-3 cm from the anterior border of the sternocleodomastoid muscle, which contracts during clenching in some individuals (Farella et al., 2008). The masseter muscle, being one of muscles of mastication, plays a major role in all functional activities of the jaw such as chewing, biting, drinking, swallowing and speaking (Farella et al., 2008). These functional

activities require coordination of the motor output elements of the masticatory muscles along with appropriate activation of tongue, facial and oropharyngeal muscles. It has been observed that the masseter is also active during non-functional activities of clenching and grinding which are considered to be risk factors for masticatory muscle pain (Huang et al., 2002; Michelotti et al., 2010).

10. SUMMARY

A number of theories have continued to evolve as knowledge accumulates concerning the relationship between pain and motor activity. Two of the most widely accepted theories; *The Vicious Cycle Theory* (Travell et al., 1942; Johansson and Sojka, 1991) and *The Pain Adaptation Model* (Lund et al., 1991) have tried to explain the interaction between pain and motor activity. There are some major issues however, with these earlier theories including that they only operate at the brainstem level (the spinal cord or segmental level for the spinal system). Also, these theories have proposed a uniform effect of pain (either excitation or inhibition, but not both) on motor activity particularly throughout a muscle, and therefore cannot fully account for the interaction between pain and motor activity. Furthermore, these theories cannot take into account the influence of higher brain centers and psychological factors (Murray G.M. et al., 2014).

Two of the more recent theories, *The Integrated Pain Adaptation Model* (IPAM) (Peck et al 2007) and *The New Theory of Motor Adaptation to Pain* (Tucker et al., 2009) have

tried to explain in detail the complex association and interaction between pain and motor activity. Both these newer models share many similarities and have endeavored to explain the interaction of motor activity and pain at different levels of the central nervous system. Many studies have been conducted in both spinal and trigeminal motor systems to determine the effects of pain on EMG activity at the level of multi-unit EMG activity with surface EMG recording electrodes (for reviews, Murray and Peck, 2007; van Dieen et al. 2003; Svensson, et al., 1996; Svensson, et al., 1997; Sae-Lee, et al., 2008).

By contrast there have been far fewer studies of the effects of pain on single motor unit (the basic functional unit) activity. An approach at the level of the single motor unit may reveal insights into the fine detail of the reorganization that might be occurring within a muscle during pain. Such fine detail may not be possible with the more global activity recorded by surface electrodes. For example, a number of studies have demonstrated that single-motor-unit (SMU) discharge rate is reduced during noxious muscle stimulation, but these studies do not explain how the force was maintained during the painful stimulation (Graven-Nielsen et al., 1997; Sohn et al., 2000, 2004; Wang et al., 2000; Farina et al., 2004, 2005; for review, see Murray and Peck, 2007). Other studies have explained this issue by demonstrating that force could be maintained by a reorganization of single motor unit activity within the muscle either as changes in the mechanical properties of the single motor units (Sohn et al., 2000, 2004; Farina et al., 2004, 2005, 2008), or through recruitment of additional SMUs within the painful muscle (Tucker et al., 2009; Tucker and Hodges, 2009).

There are number of lines of evidence showing that the recruitment of additional motor units in the presence of a reduced discharge rate or cessation of firing of motor units, can occur in pain in limb muscles (Tucker et al., 2009; Tucker and Hodges, 2009; Hodges and Tucker, 2011). Recent evidence suggests that this may also occur in the masseter muscle in experimental pain (Minami et al., 2013). This single motor unit study in the masseter muscle studied activity of single motor units at one site only in the masseter and during a single static jaw closing task only. However, the masseter muscle has a multi-pennate structure with a complex architecture and different fiber directions. It is possible that this complex reorganization that has been demonstrated at one site in the masseter may not be a generalizable feature for the entire muscle and for different jaw tasks involving the master. Furthermore, given the functional heterogeneity of the masseter muscle, there may be differences between sites as to the nature of the reorganization that occurs, if any, between the two sites. Therefore, it is anticipated that this experimental pain study will provide some insights into the effect of pain on single motor unit activity at two different sites of masseter mechanisms and could assist in the planning of future studies directed towards the management of chronic pain.

Given the above considerations, the present thesis has the following aims and hypotheses.

10.1 AIMS AND HYPOTHESIS

10.1.1 General Aims

Given the limitation of the information and conflicting findings from the previous studies as mentioned above, the general aim of our study was to determine whether experimental masseter muscle pain resulted in a change in muscle activity at 2 different sites within the masseter muscle during the performance of isometric jaw-closing tasks in asymptomatic participants.

10.1.2 Specific Aims

According to the general aims, the specific aims proposed as follows:

1. To determine whether experimental masseter muscle pain alters the ability of individuals to perform isometric jaw-closing tasks.
2. To determine whether experimental masseter muscle pain leads to changes in recruitment patterns, thresholds of firing, or firing rates of single motor units, and changes in root mean square EMG activity, within the masseter muscle during standardized isometric jaw-closing tasks and these changes occur at 2 separate sites within the masseter muscle.
3. To determine whether any changes in recruitment patterns, thresholds of firing, or firing rates of single motor units, or changes in root mean square EMG activity at one site within the masseter muscle during standardized isometric jaw-closing tasks are different to any changes occurring at another site within the muscle.
4. To determine whether experimental masseter muscle pain leads to changes in EMG activity at one or both sites within the masseter that are consistent with

earlier theories of pain-motor interaction, namely, the Vicious Cycle Theory and the Pain Adaptation Model.

5. To explore possible associations of pattern of occurrence of single motor with some psychological measures.

10.1.3 Specific Hypotheses

The following are the hypotheses of the study:

1. Experimental masseter muscle pain does not alter the ability of individuals to perform isometric jaw-closing tasks.
2. Experimental masseter muscle pain leads to changes in recruitment patterns, thresholds of firing, or firing rates of single motor units, and changes in root mean square EMG activity, within the masseter muscle during standardized isometric jaw-closing tasks
3. Experimental masseter muscle pain leads changes in recruitment patterns, thresholds of firing, or firing rates of single motor units, or changes in root mean square EMG activity at one site within the masseter muscle during standardized isometric jaw-closing tasks that are different to any changes occurring at another site within the muscle.
4. Experimental masseter muscle pain leads to changes in EMG activity at one or more sites within the masseter that are not consistent with the *Vicious Cycle Theory* and the *Pain Adaptation Model* theories of pain-motor interaction.
5. Changes in masseter muscle activity will be associated with different levels of psychological measures.

CHAPTER II

MATERIALS AND METHODS

1. EXPERIMENT DESIGN OVERVIEW, INCLUSION AND EXCLUSION CRITERIA, ETHICS

Seventeen participants (age range: 25-58 years; mean (SD): 35.12 (9.41)) without signs and symptoms of Temporomandibular Disorders or any other health problem were recruited for this study (6 males, 11 females; ages 25-58). All participants gave written informed consent prior to their inclusion in the study. Experimental procedures were approved by the Western Sydney Local Health District Human Ethics Committee and the Human Ethics Committee of the University of Sydney. Many of the procedures have been previously described in detail (Murray et al., 1999;Phanachet et al., 2001; Sae-Lee et al., 2006; Sae-Lee et al., 2008a; Sae-Lee et al.2008b; Minami et al., 2013).

Each participant completed the RDC/TMD (Research Diagnostic Criteria for Temporomandibular Disorders, Dworkin and LeResche, 1992) history questionnaire and the RDC/TMD clinical examination was performed by a calibrated examiner (Dr Terry Whittle). Inclusion criteria: unremarkable medical history (see exclusion criteria below) and Angle's class 1 occlusion, complete permanent dentition with or without third molars. Exclusion criteria: positive diagnosis following RDC/TMD history and examination, presence of one or more removable partial dentures, currently undergoing orthodontic treatment, presence of an extensive overjet and/or overbite, past history of TMD, pregnancy, high blood pressure, systemic musculoskeletal pain disorders (e.g.

fibromyalgia), systemic disease (e.g. malignancies), or medications for chronic diseases (e.g. psychiatric conditions, chronic pain conditions), or other medications that might influence a response to pain. All participants were made aware of any possible complications associated with the procedures before they signed the informed consent form. Participants were advised that they would be in pain for about 15 min according to the aims of the study and were also made fully aware that the pain would gradually diminish in a few minutes after the termination of infusion. However, participants were also informed of their right to withdraw from the experiment at any time without prejudice. The entire experimental recording session lasted about 2-3 hours.

All experimental recording sessions were conducted at the Jaw Function and Orofacial Pain Research Unit at the Westmead Centre for Oral Health, Westmead Hospital, Westmead. All participants performed standardized biting tasks on a force transducer while single motor unit activity was recorded from 2 intramuscular recording sites within the right masseter muscle. Detailed descriptions of the tasks are on pages 104-108. The tasks were repeated in 4 experimental sessions: baseline 1 (prior to any infusion), test 1 (during hypertonic saline or isotonic saline infusion into the right masseter muscle), test 2 (during isotonic saline or hypertonic saline infusion), and baseline 2 (after the infusion). The baseline 2 condition was not included in the analysis due to the possibility of post-pain effects that might influence baseline 2; such possible post-pain effects, while important, were not the main focus of this thesis. All participants performed three to six trials of biting tasks consecutively in each session of recordings. Analysis procedures

involved determining whether the induction of pain resulted in changes in the activity of single motor units at the 2 sites within the masseter muscle.

2. PSYCHOLOGICAL MEASURES

Prior to experimental recordings, each participant completed the Depression, Anxiety and Stress Scales (DASS) questionnaire (Lovibond and Lovibond, 1995). The Pain Catastrophizing Scale (PCS-13) (Sullivan et al., 1995) was completed at the end of experiment and on the basis of the experimental pain session. The McGill Pain Questionnaire (Melzack 1975) was completed by the participants after the infusions of hypertonic and isotonic saline (see below).

2.1 Depression, Anxiety and Stress Scales (DASS)

The DASS is reliable and well-validated (Lovibond and Lovibond, 1995) questionnaire which measures the cognitive and affective dimensions of psychological distress. The 3 scales of the DASS are depression, anxiety and stress (See Figure II-1). There are a total of 21 items which relate to symptoms in the past week and are rated by participants from 0 (“not at all”) to 3 (“most of the time”). The higher scores on each subscale indicate increasing liability to depression, anxiety or stress. The total scores for each scale consist of the sum of the items. This instrument was scored before the experiment.

2.2 The Pain Catastrophizing Scale (PCS)

The PCS (Sullivan, 1995) gives an assessment of the negative cognitive and affective reactions to pain. There are 13 questions that cover the 3 sub-scales of magnification, rumination and helplessness.

DASS (Lovibond & Lovibond, 1995)

Please read each statement and circle a number 0, 1, 2 or 3 which indicates how much the statement applied to you **over the past week**. There are no right or wrong answers. Do not spend too much time on any statement. The rating scale is as follows:

0 Did not apply to me at all
1 Applied to me to some degree, or some of the time
2 Applied to me to a considerable degree, or a good part of time
3 Applied to me very much, or most of the time

1	I was aware of dryness of my mouth	0	1	2	3
2	I couldn't seem to experience any positive feeling at all	0	1	2	3
3	I experienced breathing difficulty (eg. excessively rapid breathing,Breathlessness in the absence of physical exertion)	0	1	2	3
4	I tended to over-react to situations	0	1	2	3
5	I found it difficult to relax	0	1	2	3
6	I felt that I had nothing to look forward to	0	1	2	3
7	I felt that I was using a lot of nervous energy	0	1	2	3
8	I felt I wasn't worth much as a person	0	1	2	3
9	I felt that l was rather touchy	0	1	2	3
10	I felt scared without any good reason	0	1	2	3
11	I found it hard to wind down	0	1	2	3
12	I was aware of the action of my heart in the absence of physical exertion (eg, sense of heart rate increase, heart missing a beat)	0	1	2	3
13	I felt down hearted and blue	0	1	2	3
14	I felt I was close to panic	0	1	2	3
15	I was unable to become enthusiastic about anything	0	1	2	3
16	I was intolerant of anything that kept me from getting on with what I was doing	0	1	2	3
17	I felt that life was meaningless	0	1	2	3
18	I found myself getting agitated	0	1	2	3
19	I was worried about situations in which I might panic and make a fool of myself	0	1	2	3
20	I experienced trembling (e.g., in the hands)	0	1	2	3
21	I found it difficult to work up the initiative to do things	0	1	2	3

Figure II-1 The Depression, Anxiety and Stress Scale (DASS-21).

Pain Catastrophizing Scale

Sullivan MJL, Bishop S, Pivik J. (1995)

Everyone experiences painful situations at some point in their lives. Such experiences may include headaches, tooth pain, joint or muscle pain. People are often exposed to situations that may cause pain such as illness, injury, dental procedures or surgery.

Instructions:

We are interested in the types of thoughts and feelings that you have when you are in pain. Listed below are thirteen statements describing different thoughts and feelings that may be associated with pain. Using the following scale, please indicate the degree to which you have these thoughts and feelings when you are experiencing pain.

RATING	**0**	**1**	**2**	**3**	**4**
MEANING	Not at all	To a slight degree	To a moderate degree	To a great degree	All the time

When I'm in pain …

Number	**Statement**	**Rating**
1	I worry all the time about whether the pain will end.	
2	I feel I can't go on.	
3	It's terrible and I think it's never going to get any better	
4	It's awful and I feel that it overwhelms me.	
5	I feel I can't stand it anymore	
6	I become afraid that the pain will get worse.	
7	I keep thinking of other painful events	
8	I anxiously want the pain to go away	
9	I can't seem to keep it our of my mind	
10	I keep thinking about how much it hurts.	
11	I keep thinking about how badly I want the pain to stop	
12	There's nothing I can do to reduce the intensity of the pain.	
13	I wonder whether something serious may happen.	

Figure II-2 The Pain Catastrophizing Scale (PCS).

These subscales capture a person's orientation towards noxious stimuli and/or previous memories of pain. Each of the 13 questions is rated on a 5-point Likert scale ranging from 0 (not at all) to 4 (all the time), with the total score ranging from 0-52.

The PCS (Figure II-2) was completed after the experiment and participants were instructed to consider the noxious muscle stimulus in the experiment. This is because many of our participants were young, healthy adults with limited pain experience. It was therefore thought that it is best to use a PCS score based on the pain experienced at the actual experimental pain session.

2.3 The McGill Pain Questionnaire (MPQ)

The MPQ (Melzack, 1975) consists of 3 major classes of word descriptors — sensory, affective and evaluative — that are used by participants to describe their subjective pain experience. The main measures are: (1) the pain rating index, (2) the number of words chosen, and (3) the present pain intensity. Volunteers completed the MPQ by recalling their pain experience immediately after the pain had declined to zero and following the cessation of the hypertonic saline infusion. The MPQ was also completed after the infusion of the isotonic saline to describe the subjective pain experience, if any, associated with isotonic saline infusion and therefore to allow a comparison between the 2 infusions of the subjective experience.

3. AREA OF PAIN SENSATION

After completion of each saline infusion, each participant marked the location and the maximum distribution of perceived pain on right and left lateral-profile outline pictures of the head and neck (Sae-Lee et al., 2008). Pain referral sites on the pain maps were also noted. A pain referral site was defined as a region that was separate from and did not overlap with the area of pain spread outlined and surrounding the injection site. No participants reported referral sites outside the head and neck region. After the termination of infusion, the effect of pain was enumerated with the McGill pain questionnaire (MPQ).

3.1Visual Analogue Scale

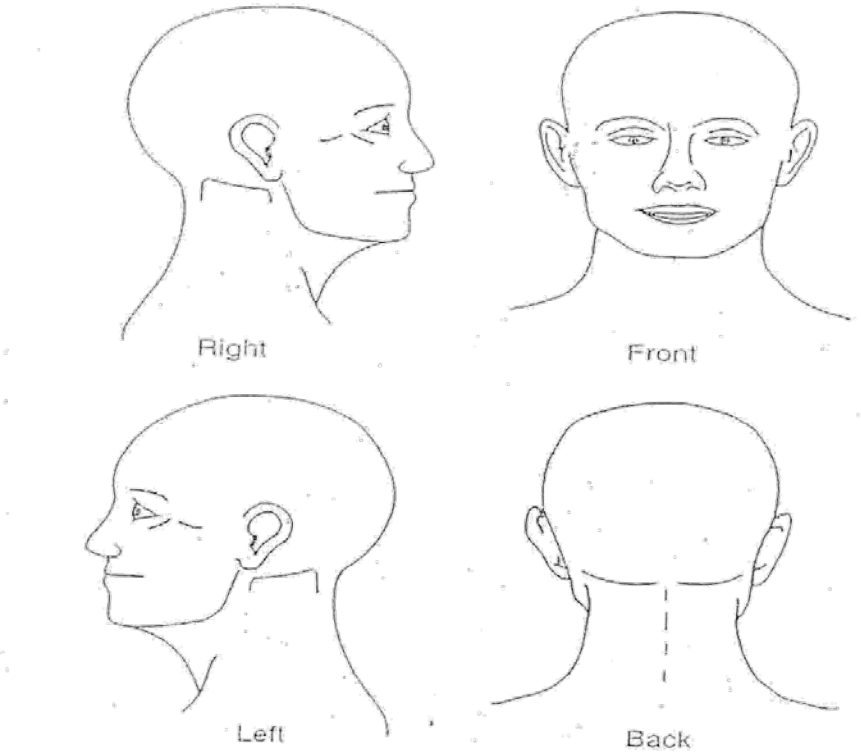
In the infusion trials, participants rated the perceived pain intensity on a 100-mm visual analogue scale (VAS; Figure II-3). The scale was anchored between 0 and 100, the lower endpoint was marked ‘no pain at all’ and rated ‘0 mm’ whereas the upper endpoint was marked ‘the worst pain imaginable’ and rated ‘100 mm’. The evoked pain was rated after the insertion of the infusion catheter, before instigation of infusion and then after the completion of each jaw task trial during both saline infusions. During the hypertonic saline infusion, the pain intensity was maintained at 30-60/100 by adjusting the infusion rate of the hypertonic saline (for details see page-110).

TRIAL NUMBER :_____

- PUT A CROSS ON THE LINE TO SHOW **HOW STRONG YOUR PAIN** IS

NO PAIN _____ WORST PAIN POSSIBLE

- DRAW THE AREA ON THE PICTURE TO SHOW **WHERE YOU FEEL PAIN**



- MARK THE AREA INSIDE YOUR MOUTH **IF YOU FEEL PAIN**

Area		Specific Side			
		Left	Right	Front	Back
Teeth					
Gum					
Tongue					
Palate					
Other		Please specify:			

Figure II-3 The Visual Analogue Scale (VAS) and pain maps that were used in the second and third sessions where hypertonic saline or isotonic saline was infused into the right masseter muscle.

4. JAW MUSCLE EMG RECORDING

A topical anesthetic gel (Emla®, Aatra, Australia) was applied at the site of placement over the masseter muscle of the fine-wire electrodes 15-20 minutes before inserting the electrodes into the muscle, in order to avoid local skin pain. Bipolar fine-wire electrodes made of Teflon®-coated stainless steel wire (diameter 0.0045 mm) were inserted at 2 sites within the right masseter muscle via a 25-mm-long needle (Sae-Lee et al., 2006; Uchida et al., 2001). In 3 of the participants, the insertion sites were arbitrarily assigned to the anterior and the posterior part of the muscle, and in the remaining 14 participants, the insertion sites were in the superior and inferior parts of the muscle (for rationale see page-116). Prior to the placement of both electrodes, the muscle was palpated by asking the participant to clench and the outline of the masseter muscle boundaries was drawn with a non-indelible marker.

In the 14 participants where the insertion sites were approximately in the superior and inferior parts of the palpable masseter, the location at which the fine-wire electrodes were inserted into the masseter muscle was guided after placement of the splints in the mouth (see below) by asking the participants to gently clench so as to activate the jaw closing muscles. The contraction of the masseter was palpated and the region where the masseter appeared to be contracting to its maximum amount was determined in all 14 participants to be in the superior and inferior parts of the muscle. These regions were therefore taken as the regions where each needle was inserted into the masseter muscle. The purpose of this procedure was to determine the most optimal position for electrode placement and where muscle activity was most likely to be recorded. Immediately prior to placement of each needle electrode, the skin surface was wiped with 70% isopropyl alcohol (Alco

wipe, Pro medica, Australia). The needle was inserted ~20 mm beneath the skin or until bone was contacted, and then the needle was withdrawn, leaving the fine wires within the muscle. Although mild pain was experienced on intramuscular electrode placement, no participant was in pain several minutes after electrode placement. After placement of one of the fine-wire electrodes, then the second fine-wire electrode was placed. There was no predetermined order to the placement of the intramuscular electrodes within the masseter.

The EMG activity was amplified (2,000x-10,000x), filtered and digitized (Model DBA-S, World Precision Instruments Ltd, UK). The sampling rate was 20,000 samples/s for the recordings using fine-wire electrodes and 5,000 samples/s for the surface EMG recordings (Model micro1401, Cambridge Electronic Design (CED), Cambridge, England). The bandwidth was 20 Hz to 1 kHz for the surface recordings and 100 Hz to 10 kHz for the fine-wire recordings. A ground electrode was attached to the left wrist .

4.1 Jaw Tasks and Recording of Bite Force

Bite force was measured with a force transducer secured to an acrylic resin splint surrounding the upper dentition (Figure III-4 and III-5). A metal ball was attached to the unit of the force transducer via a hinged plate. The metal ball contacted another metal plate that was attached to another resin splint covering the lower anterior teeth (Figure III-4 and III-5) and this lower metal plate was oriented parallel with the occlusal plane at the lowest possible vertical dimension. When the ball contacted the lower plate, the vertical dimension was 5 – 8 mm open from the intercuspal position because of the

thickness of the splints and the placement of the sensor. Participants were instructed to close lightly so that the ball contacted the lower plate and at this posture mandibular position was 2-3 mm open from the postural jaw position. As the lower plate was oriented parallel with the occlusal plane, it was considered that the jaw should slide horizontally when the applied force direction changed from being perpendicular to the occlusal plane. None of the participants commented that the ball had moved on the horizontal plate at any stage during the experiment. This arrangement helped ensure that all participants would direct each application of closing force as closely perpendicular as possible to the occlusal plane. The lower metal plate was marked with marker pencil prior to each biting task in each participant. This allowed confirmation of the stability of the contact position between the ball and the lower plate throughout each biting task. At the beginning of each biting task, each participant was instructed to lightly close the jaw so that the metal ball just contacted the lower plate (Figure II-4) so that there was zero force output from the transducer at this position.

The isometric force signal, derived from the transducer, was recorded using the AMLAB data-acquisition system (Associative Measurements, North Ryde, Sydney, Australia) at a sampling rate of 1000 samples/s and bandwidth of 0–500 Hz. The output from the AMLAB system was also displayed on a computer screen positioned in front of the participant. The force output from the force transducer was recorded with a different computer to the computer that was used to record the intramuscular EMG. The use of separate computers created some initial difficulty with the analysis procedures because the 2 sets of data (force data and the EMG data) had to be synchronized.

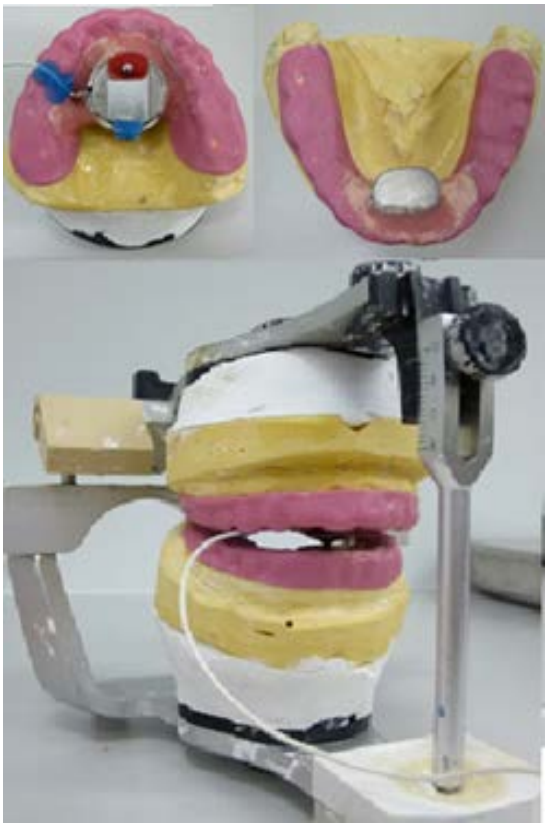


Figure II-4 Upper and lower models with acrylic splints and force transducer attached to the upper splint.

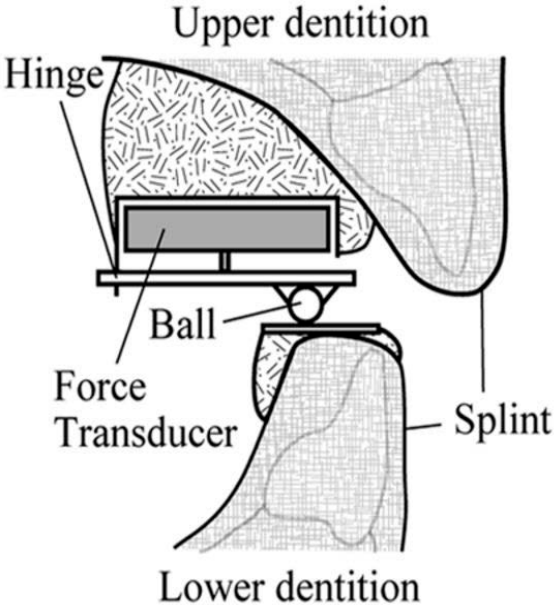


Figure II-5 Experimental apparatus showing the method of measuring bite force and controlling force direction. A force transducer (LM-5KA; Kyowa Dengyo, Tokyo, Japan) was secured to an acrylic resin splint surrounding the upper dentition, a metal ball attached to a hinged plate covering the force transducer. The metal ball was in contact with another metal plate secured to another resin splint covering the lower anterior teeth, and this lower metal plate was oriented parallel to the occlusal plane at the lowest possible.

This synchronization resulted in the data files starting at the same time point. In the actual recording session, the acquisition of the force data with the AMLAB computer was commenced prior to the acquisition of the EMG data with the Cambridge Electronic Design Limited, Cambridge, UK (CED) system. The synchronization of the data files is described in the Data Analysis section.

The recording of Bite force and jaw muscle EMG activity was carried out in a repeated-measures design consisting of the following four sessions:

1. Baseline 1 session (before any infusion),
2. Test 1 session (during hypertonic or isotonic saline infusion),
3. Test 2 session (during isotonic or hypertonic saline infusion),
4. Baseline 2 session (after test 1 and 2).

Each participant was instructed to carry out 2 biting tasks (ramp biting task and step level biting task) during each recording session (as described above) with force transducer in the mouth. The performance by the participants of one of the following tasks was termed a “trial”.

1. Ramp biting task.

   For recording of ramp task, the force level was displayed as a target line on a computer screen in front of the participant to provide a visual feedback. Each participant was asked to initiate a contraction and to follow the computer controlled target by progressively increasing the force. The rate of force increase was standardized across all participants at 21.5 N/s. Each ramp trial task consisted of 15 seconds of following the target followed by cessation of biting and a 15 second rest period. Each participant was instructed to match the target force level

as closely as possible during 15 second recording period. Three trials of ramp were taken under each baseline (1 and 2) and test sessions (hypertonic and isotonic infusion sessions).

2. Step level biting task

   The step levels for this task were customised to each participant in the following way. Each participant was instructed to increase their bite force until a single or a small number (e.g. 2-3) of single motor units were visible in the computer screen displays of both intramuscular EMG channels. The force level at which these units became active was taken as the first target force (step) level. Then each participant was instructed to further increase their bite force so that the existing single motor units increased their firing rates or more single motor units were recruited. The 2nd bite force level was selected at the force level where there was at least an increase in firing rate of SMUs that were already active at the 1st step level. This assessment was carried out qualitatively at the time of the experiment. This higher force level was taken as the second target force (step) level. Both target force (step) levels were marked on the participant's computer screen for visual feedback.

   In the first 3 participants only the recording of a single step biting task (Target level 1) was undertaken whilst in the other 14 participants recording of 2 step biting tasks (target level 1 and 2) was performed. Each trial of a step level biting task consisted of a rest period of 2 seconds (~2 s), followed by biting and holding at the 1st target force level for 2 to 3 seconds (2-3 s), then increasing the biting force to achieve the 2nd target force level and holding at this 2nd target force level

for 2 to 3 seconds (2-3 s), followed by cessation of biting and return to rest for 2 seconds (~2 s) (see Figure II-6).

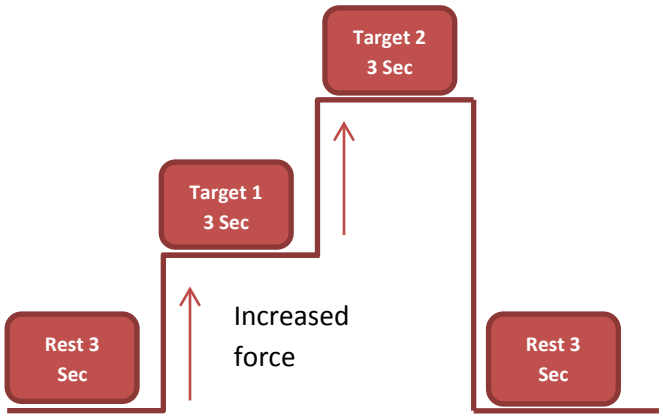


Figure II-6 A brief description of recording of each trial of step level biting task with recording time.

The participants were encouraged and instructed to maintain bite force as close as possible to the target force level during the 1st and 2nd target force levels. Three to six trials were performed consecutively in each session of recordings; see above for definition of a session of recordings. While different participants exerted different forces at each of step level 1 and step level 2, the same force levels selected within a participant were used for all sessions in that participant, namely, baseline, isotonic and hypertonic saline infusion.

4.2 Induction and Assessment of Jaw-Muscle Pain

Some of the study design and procedures for experimental jaw-muscle pain induction were similar to those previously described in detail (Sae-Lee et al., 2006; Sae-Lee et al.,2008b). Figure II-7 shows the experimental setup in one participant. Experimental pain was induced by the tonic infusion of 5% hypertonic saline into the deep central region of the right masseter muscle (Figure II-7) which has previously been documented to induce masseter pain in humans (Svensson et al., 1997, 1998b, 1999; Stohler et al., 1992; Sae-Lee et al., 2008 a, b). A disposable 24-gauge needle integrated IV catheter (JELCO, 22 G x 1", Smiths Medical ASD, Inc. Southington, CT 06489 USA) was inserted into the right masseter muscle (Figure II-8). The needle was retracted at bone contact or at 2 cm depth if there was no bone contact and the catheter remained in the muscle. A 1 ml syringe (Becton Dickinson, Singapore) was then attached to the needle and negative aspiration (to ensure no infusion of saline has entered into blood) was carried out before injection of the saline.

The catheter was connected to an extension set (extension set with polyethylene inner line, 75 cm, 0.7 ml) and a 10 ml syringe secured into an infusion pump (IVAC Model P2000, UK). The syringe was filled with 5% sterile hypertonic saline for the hypertonic saline infusions or 0.9% sterile isotonic saline for the isotonic saline infusions. Participants were given standardized instructions and were not told which solution was about to be injected (single blind), although they quickly became “un-blinded” particularly if the hypertonic saline was infused first. If hypertonic saline was infused first, then a bolus infusion of 0.2 ml 5% hypertonic saline was infused over 20 s to achieve rapidly a pain intensity of between 30–60 mm that was denoted by the participant on the 100-mm visual analog scale (VAS).

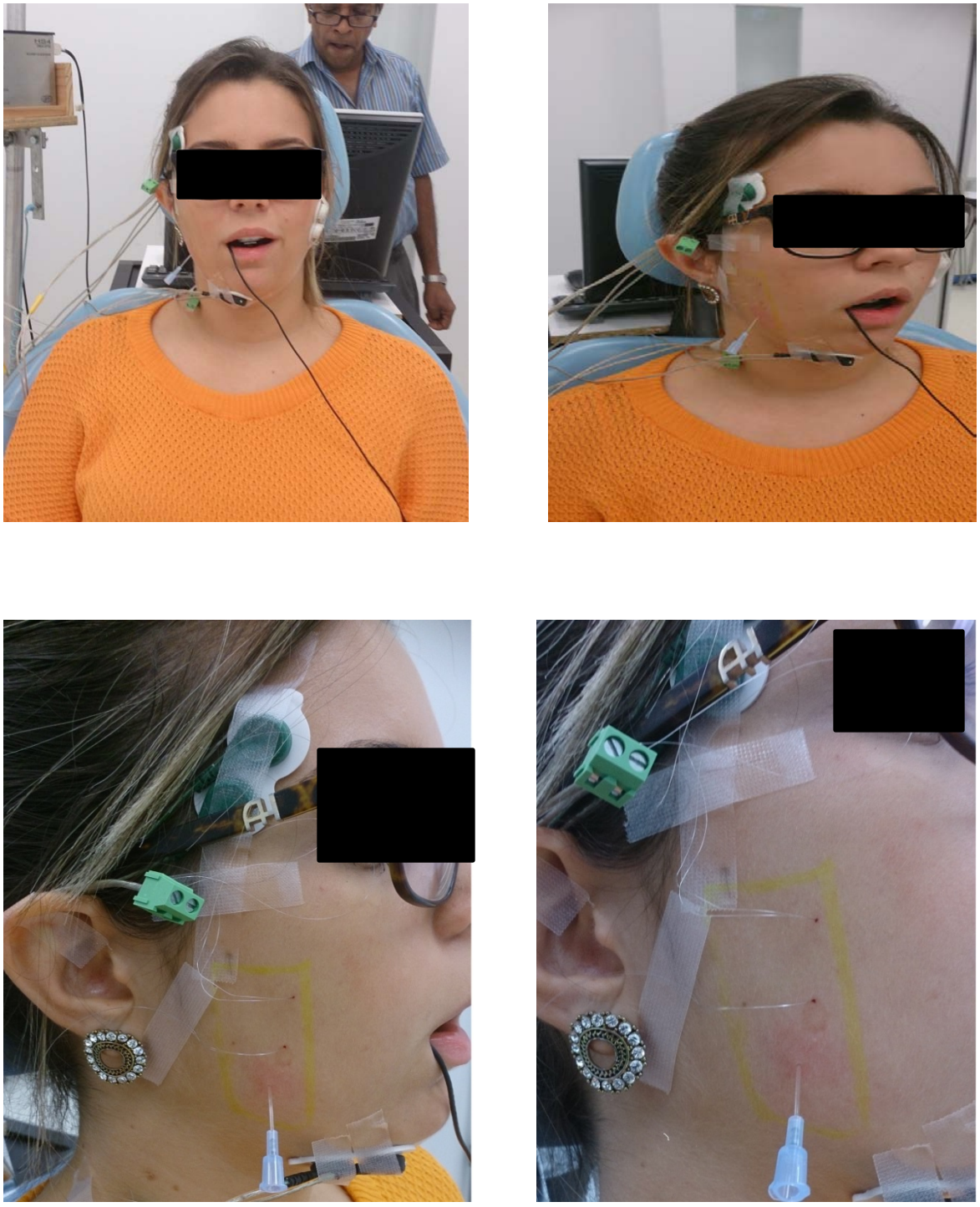


Figure II-7 The front view of a participant showing experimental setup in right masseter muscle pain study with upper and lower splints and surface electrodes in place (A). The lateral view showing the plastic catheter inserted in the thickest part of the masseter muscle (B). A closer view of the lateral profile of the participant with the fine-wire electrodes placed in the right masseter muscle and the catheter in place (C, D). Note that the activity from the surface EMG electrodes was not analysed in this thesis.

Then, continuous infusion was maintained by the infusion pump with a steady infusion rate of 2–9 ml/h during each session. Pain intensity was quantified on the VAS prior to catheter insertion, after insertion but prior to the commencement of infusion, and at every 30 s after commencement of infusion until pain ratings were between 30 and 60 mm on the VAS, and then after each biting task trial. At each of these times, a single VAS score was obtained. Manual changes in infusion rate were made in steps of 1-3 ml/h to maintain pain intensity at a constant level of 30–60 mm on the VAS.

The catheter was inserted into the masseter muscle immediately after completion of baseline 1. The isotonic saline infusion was defined as the no or minimal pain session; the hypertonic saline infusion was defined as the pain session. The time between the end of test 1 and the beginning of test 2 was 10 min and this was sufficient time to allow any pain from the previous infusion to subside to 0.

The same infusion procedure used for hypertonic saline infusion was used for 0.9% isotonic saline infusion. The order hypertonic saline first, isotonic saline second was alternated between participants. Thus the isotonic saline condition (i.e. no or minimal pain condition) served as a control in each of the 17 participants for possible bite force and/or EMG effects from volumetric change within the muscle of the infused solution. With this standard protocol, pain intensity levels were able to be kept relatively constant throughout the infusion period. The volume of hypertonic saline that was infused was determined by the need to achieve the target VAS score of 30-60/100. If the isotonic saline was infused first (the order of infusion of each solution was alternated between participants), the rate for the isotonic saline infusion was set at a rate of 4 ml/h for 5 minutes and then increased to 6 ml/h for the last 5 minutes. If isotonic saline was infused

second, the rate of infusion of the isotonic saline was adjusted to follow the rate of the previous hypertonic saline infusion in that participant. In some of the participants (9 participants) the infusion catheter was placed below the both intramuscular EMG electrodes and in some participants (8) the catheter was placed between the two intramuscular EMG electrodes and the reason for this variance was the difference in most optimal position for electrode placement (muscle activity) in each participant . All jaw tasks were performed in a single sitting on a single experimental day. As indicated above, the McGill Pain Questionnaire (MPQ) was completed by each participant after the hypertonic saline infusion and the isotonic saline infusion was terminated to estimate the sensory-discriminative, affective, and evaluative components of the pain experience. There were no complications and this was consistent with previous reports (Sae-Lee et al., 2006; Sae- Lee et al., 2008b; Svensson et al., 1997).

Below is a summary of whole experiment, including its sequence of recording sessions along with questionnaires given to the participants. Note each session consists of three recordings of ramp tasks and 5-6 recordings of step tasks.

In first visit detailed description of procedure to the participant, signing the Consent form and impression taking for making splints. At the day of experiment RDC/TMD and DASS, getting all the setup ready for the experiment, placement of electrodes, recording of first session (baseline 1), placement of catheter and recording of second session (test 1 Hyper/Iso), VAS after each trial and MPQ after termination of infusion, recording of 3rd session (Test 2 Hyper/Iso), VAS after each trial and MPQ after termination of infusion, recording of 4th session (baseline 2), removal of electrodes.

5. DATA ANALYSIS

Participant demographic data was explored and described either qualitatively or quantitatively. Independent samples t tests were performed on the scales of the DASS, the PCS and the VAS to explore gender differences between the variables. Weighted scores were calculated for each of the pain descriptors from the MPQ (Melzack, 1975) and presented qualitatively to describe the computed value of the pain experience.

The intensity of the experimental pain was measured by the VAS after each jaw task trial within each repeated task within each session, that is, baseline and, hypertonic or isotonic saline infusion. The data was tested for differences between the repetitions of a task with a Repeated-Measures Analysis of Variance (ANOVA) to enable task means of the repeated tasks to be used in further analyses. The difference in the level of perceived pain intensity between the hypertonic and isotonic saline infusions for each of the tasks was determined from the mean of the repetitions with paired t-tests. Paired t-tests were also used to determine a difference in volume of saline infused between the hypertonic and isotonic saline sessions. The data were normally distributed (see Results) and therefore parametric tests were used for the analysis.

As the two data acquisition computers commenced recording data at different times, data for each trial was synchronized to determine the start of each recording. The time point at which the Spike 2 (v7.06) software (Cambridge Electronic Design Limited, Cambridge,

UK) tagged the EMG tracing with the change from resting to activation level of the force transducer was taken as zero and the following time points adjusted accordingly. The time point at which the force data (AMLAB computer) changed from the resting level to activation was taken as zero and the following time points adjusted accordingly.

Single motor unit (SMU) EMG activity at both sites, superior/anterior and inferior/posterior (RMS/RMA,RMI/RMP) within the right masseter muscle was analyzed with Spike 2 (Cambridge Electronic Design Limited, Cambridge, UK) software during the ramp biting task and the step level biting task under baseline 1 and test sessions. The data collected during the baseline 2 session were not analysed in this thesis. The effect of experimental jaw muscle pain on the motor unit recruitment, firing thresholds and firing rates was then quantitatively explored between baseline and test 1 and test 2 sessions.

The force at which each participant carried out the ramp and the step tasks was extracted in volts and converted to Newtons (N) using the following formula:

Load (N) = (Bridge output/gain) x rated capacity of the transducer/rated output (mV/V)

The converted force values, recorded at 1000 samples/second, were graphed using the mean and standard deviation of every 500 ms epoch throughout the entire recording trial. The graphs were used to identify the start and end points of each of the ramp and each

level of the step tasks, and to determine the least variable (most stable) period during the step tasks.

In the following analysis, single motor unit activity was initially analyzed at each site within the masseter muscle separately and reported separately. After an initial analysis of data at each site, then a further analysis was performed comparing the activity of SMUs between sites.

For the purposes of the analysis, the data from the participants where recordings were made from the anterior intramuscular masseter site (n=3) were combined with the data from the participants where recordings were made from the superior intramuscular masseter site (n=14). This was then termed the superior/anterior site (RMS/RMA site). The data from the participants where recordings were made from the inferior intramuscular masseter site were combined with the data from the participants where recordings were made from the posterior intramuscular masseter site. This was then termed the inferior/posterior site (RMI/RMP site). The rationale for combining the superior with the anterior site and the inferior with the posterior site data was partly arbitrary and related to convenience of the analysis, but also partly based on the anatomy of the masseter muscle. It was felt that, given that the masseter is inclined anteriorly towards its superior aspect, combining the superior with the anterior site and the inferior with the posterior site data was reasonable.

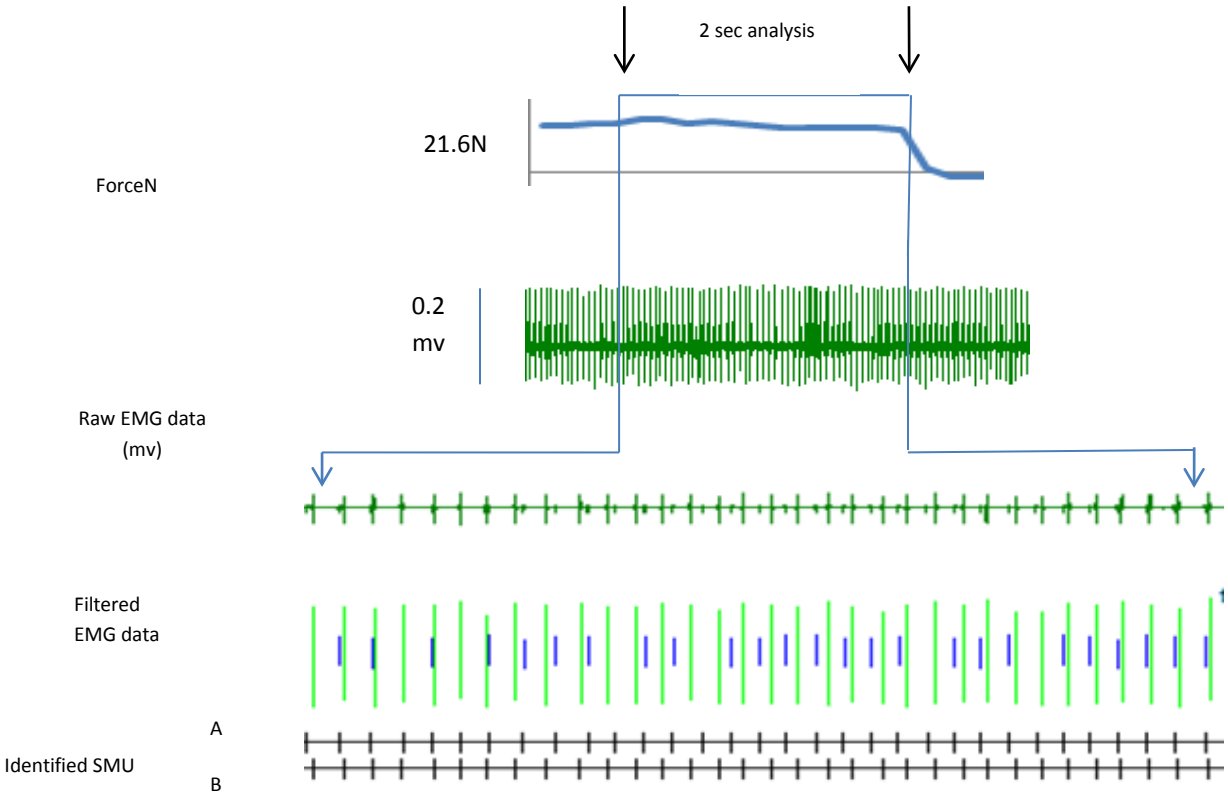


Fig III-8 Upper 2 traces show force and EMG recording from the right masseter muscle during isometric biting task (step 2). The 2-second analysis period where the force was stable is indicated by the arrows and the vertical lines. The concomitant section of EMG is shown in expanded form in the lower panel, where after filtering EMG data 2 single motor units have been discriminated (A, B) using spike 2 software.

Muscle activity during each of the tasks from each of the recording sites in the right masseter muscle was established by discriminating individual SMUs from the intramuscular EMG recordings from the masseter muscle. The criteria for SMU

definition were close similarities in amplitude and waveform between all representatives of an identified SMU by automated template matching and confirmed by visual inspection. Spike 2 (v7.06) software (Cambridge Electronic Design Limited, Cambridge, UK) was used to carry out the definition of individual SMUs. In the ramp task, a SMU was included for analysis if it could be clearly identified in at least 1 trial of the task in that session and it was clearly able to be identified as present or not present in subsequent trials of that session and in other sessions. With higher forces in the ramp task, the recruitment of additional SMUs was difficult to identify clearly and therefore these SMUs were not included in the count of SMUs. Clear identification of the presence or not of a particular SMU was only possible during the lower forces of the ramp task.

Figure III-8 gives an overview of how the SMUs have been discriminated after synchronizing the force and EMG data using the 2 second stable period (see above).

For the step level task, SMU discrimination followed a similar process to that in the ramp task where a SMU was only included in the analysis if it could be clearly identified as being present or not present in all task trials in that participant. The time period of 3 seconds in the 1st step level and 2nd step level where the force levels were least variable was chosen as a stable period. The stable periods were established by calculating the mean and standard deviations of each 500 millisecond of Newton force data and graphed for visual inspection. The time period of at least 1 second with the least variability from the mean force, that is, standard deviation was selected as the stable period of that step

level. The mean force level (the mean of 5 trials of each session) at the 1st and 2nd step levels in the 2 step task in each session were calculated for each participant. A comparison of mean force level across sessions within each step level was carried out with repeated measures ANOVA to determine if the force between the sessions was not significantly different.

Each SMU from each intramuscular recording site that could be clearly identified, according to the criteria above, where then entered into an Excel spreadsheet that indicated whether that SMU was active or not active during each jaw task trial in each session. This analysis allowed an assessment of whether the SMU was active in each trial of all sessions or only some trials or sessions. For example, this analysis allowed a determination whether a SMU was recruited (i.e. become active) during a session or de-recruited (i.e. become inactive) during a session. Further analyses were done of the occurrences of SMUs during the tasks between the 2 intramuscular recording sites and statistical tests involved a Chi-square test. An analysis was also performed to determine whether the changes in the pattern of occurrence of SMUs at the two sites within the masseter muscle were consistent with the Vicious Cycle Theory or the Pain Adaptation Model.

Each identified SMU at each site was further analysed by ascertaining the time point in each recording trial at which the SMU commenced firing continuously. It was clearly apparent that once a SMU commenced firing in the ramp task, it continued to fire and to increase its firing rate in association with the increase in ramp force. This time point at

which a SMU commenced firing was matched to the force data and a threshold value in Newton's was therefore assigned to each discriminated SMU for each trial in which it was activated. A SMU threshold value was therefore able to be determined for the ramp task, and also for the same SMU, a threshold value was able to be assigned for the 2-step task in a similar manner, that is, a SMU commenced firing at some stage in the 2 step task, and it continued to fire and to increase its firing rate in association with the increase in force.

SMU threshold values were used to determine if experimental muscle pain changed the activation pattern of SMUs within either of the recording sites of the right masseter muscle, or for either of the ramp or step tasks. Statistical analyses consisted of Univariate ANOVA to determine effects of the repetitions within each of the tasks and to explore differences in threshold values between the infusion sessions for each of the ramp or step tasks. Differences in threshold values within the participants and between recording sites was explored using a repeated measures ANOVA that tested the effect of session and the interaction of the sessions with the two right masseter sites.

In order to capture the differences in the EMG activity for all of the participants, the data was re-analyzed using the root mean square (RMS) of the EMG signal at each intramuscular masseter EMG site. The RMS is the square root of the average of the squared values within a time period and provides a standardised level of muscle activity for comparison within and between participants. In this form of processing, the EMG

signal is submitted to calculations that are designed to quantify the intensity and the duration of several events of the EMG signal (Fridlund et al., 1986, 1995).The RMS value is, therefore, a parameter frequently chosen because it reflects the level of the physiological activities in the motor unit during contraction (Fukuda et al., 2008).

In this instance, the time periods were the time between the start and finish of the force applied for the ramp task and the start and finish of each stable period of the levels of the step task as previously described. Statistical analyses, repeated-measures ANOVA, was run on the 3 repetitions of the ramp task and 5 repetitions of the step tasks of 17 participants to determine whether there was a significance effect of repetition in RMS activity. If no effect is identified, a mean RMS for each of the intramuscular masseter sites across the force tasks will be calculated to determine if there is an effect of session.

In order to confirm the discrimination accuracy, motor unit inter-spike intervals were examined using the same stable period in step level biting tasks (See above). Firing rates of 20 discriminated SMU's were calculated by obtaining the number of times a SMU was active over a period of 1 second. For analysis instantaneous firing frequencies of each SMU were qualitatively compared between the hypertonic and isotonic recording sessions. All statistical analyses were undertaken using Statistical Package for the Social Sciences Version 22 (SPSS v22) and a level of probability of less than 0.05 ($p < 0.05$) was taken as a significant result.

CHAPTER III

RESULTS

1. PARTICIPANTS

Seventeen asymptomatic participants with an age range of 25-58 years participated in this single motor unit experiment (Table III-1). Of the 17 participants, 6 were male and 11 were female. Fourteen participants completed both the ramp and step level biting (force) tasks and the remaining 3 participants completed the step (level 1) tasks only (Table III-1). There were no drop-outs from the study.

Table III-1 Participant demographics listing gender, age and task completed.

Participant	Gender	Age	Ramp task	Step task
S1	F	31	No	Yes
S2	F	30	No	Yes
S3	F	37	No	Yes
S4	F	31	Yes	Yes
S5	M	32	Yes	Yes
S6	M	37	Yes	Yes
S7	F	35	Yes	Yes
S8	M	34	Yes	Yes
S9	M	32	Yes	Yes
S10	F	30	Yes	Yes
S11	F	38	Yes	Yes
S12	M	40	Yes	Yes
S13	F	34	Yes	Yes
S14	F	25	Yes	Yes
S15	F	37	Yes	Yes
S16	M	58	Yes	Yes
S17	F	26	Yes	Yes

2. EXPERIMENTAL MASSETER MUSCLE PAIN

2.1 Results of RDC/TMD

The Research Diagnostic Criteria for Temporomandibular Disorders (RDC/TMD) (Dworkin and LeResche, 1992) was performed by a calibrated examiner to determine any signs or symptoms of TMD and neuromuscular dysfunction in each participant. Out of all of the participants, 3 reported jaw clicking/popping, 1 gave a history of jaw grating/grinding, 5 reported clenching of teeth during the night time and 1 reported grinding during the day time. Out of 17 participants, 2 reported a family history of arthritic disease, 1 reported a history of headache and 1 reported problems with eating hard food. None of the participants reported muscle soreness on palpation of the right or left jaw muscles which included the temporalis, masseter and submandibular muscles.

2.2 Outcome of Psychological Questionnaires

Although possible gender differences were not the main focus of analysis, we ran a simple t-test to examine for possible gender differences for the psychological variables (DASS and PCS) and for the pain intensity score (i.e. VAS).

2.2.1 DASS

A list of the scores for depression, anxiety and stress for each participant is shown in Table III-2.

Table III-2 Depression, Anxiety and Stress (DASS) scores for each of the participants and mean and standard deviation (SD) of each of the scale scores

Participant	Depression	Anxiety	Stress
P1	1	0	2
P2	0	0	0
P3	0	0	0
P4	0	0	0
P5	1	4	6
P6	3	3	4
P7	0	0	0
P8	1	0	0
P9	4	2	0
P10	0	0	0
P11	3	3	3
P12	0	0	0
P13	0	0	0
P14	0	3	0
P15	0	0	0
P16	2	0	1
P17	0	0	0
Mean	**0.88**	**0.88**	**0.94**
SD	**1.32**	**1.45**	**1.78**

All of the participants in the current study had low scores for each of the scales, depression, anxiety and stress, and the mean scores for each scale were all below 1 (Table III-2). The self-report of depression was found to be significantly different ($p = 0.02$) between the male 1.8(SD: 1.5) and female 0.4(SD: 0.9) participants, although anxiety ($p = 0.21$) and stress ($p = 0.25$) were not significantly different.

2.2.2 PCS

Pain catastrophising scores for each participant are shown in Table III-3. This table shows the total score and scores for each of the 3 sub-scales as well as means and standard deviations of all of the participants' scores. The table shows that pain-related catastrophizing varied considerably between the participants with a total score range of 0 to 33 out of a possible score of 52. Table III-3 also shows that the mean total score was low at 9.5 (SD: 9.0) and that within the 3 subscales, rumination had the highest group mean (4.2) followed by helplessness (3.6) and magnification (1.7).

There were no significant differences between males and females for the total score ($p = 0.05$), rumination ($p = 0.11$), magnification ($p = 0.73$) or helplessness ($p = 0.06$).

Table III-3 Pain catastrophising scale (PCS) scores for each participant showing total score and scores for each of the 3 sub-scales as well as means and standard deviations of all of the participants' scores.

Participant	Rumination	Magnification	Helplessness	Total Score
P1	2	1	0	3
P2	4	11	2	17
P3	1	3	0	4
P4	2	0	3	5
P5	13	4	16	33
P6	3	1	10	14
P7	0	0	0	0
P8	0	0	2	2
P9	16	0	6	22
P10	6	1	8	15
P11	5	1	7	13
P12	3	2	0	5
P13	1	0	1	2
P14	4	2	1	7
P15	0	0	0	0
P16	10	1	4	15
P17	2	1	1	4
Mean	**4.2**	**1.7**	**3.6**	**9.5**
SD	**4.6**	**2.7**	**4.5**	**9.0**

Figure III-1depicts the differences in mean scores between the genders.

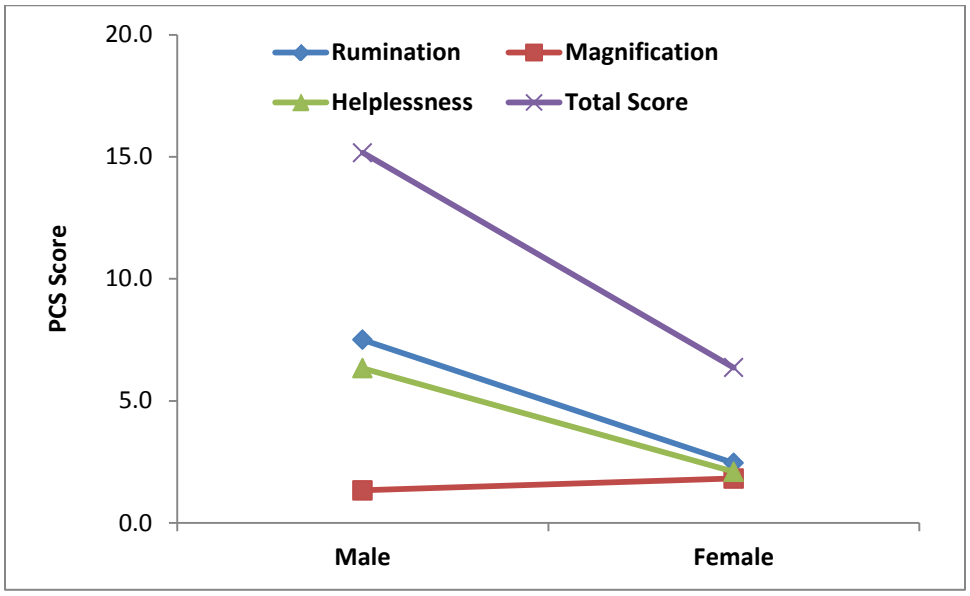


Figure III-1 Mean Pain Catastrophizing scores for the Total Score and the 3 subscales for male and female participants.

2.3 Sensory Experience of Experimental Right Masseter Muscle Pain

2.3.1 Infusion Parameters

Table III-4 demonstrates the total volumes (ml) of hypertonic and isotonic saline solutions infused in each participant. The mean and standard deviation of the total infused volume across the 17 participants who were able to accomplish the required jaw tasks (ramp and step) during hypertonic saline and isotonic saline infusion was 0.9 (SD: 0.5) and 1.0 (SD: 1.4) ml, respectively.

Table III-4 The total volume (ml) of infused solutions in each participant and the mean and standard deviation (SD) for each solution and the order of infusion. The order of infusion was alternated between most of the participants.

Participant	Gender	Volume (ml)		First Infusion
		Hypertonic saline	Isotonic Saline	
P1	F	0.7	0.6	Hyper
P2	F	0.9	0.4	Iso
P3	F	0.9	0.6	Hyper
P4	F	1.1	0.6	Iso
P5	M	0.4	0.4	Hyper
P6	M	0.6	no data	Hyper
P7	F	0.8	0.8	Iso
P8	M	1.5	1.3	Hyper
P9	M	1.6	1.0	Iso
P10	F	0.5	0.1	Iso
P11	F	0.6	6.0	Hyper
P12	M	0.6	0.5	Iso
P13	F	0.8	0.5	Hyper
P14	F	0.6	0.5	Iso
P15	F	0.8	0.7	Hyper
P16	M	2.3	1.5	Hyper
p17	F	0.4	0.4	Hyper
Mean		**0.9 ml**	**1.0 ml**	
SD		**0.5 ml**	**1.4 ml**	

No data = Infusion had not been finished in this participant due to needle instability and the unwillingness of the participant to have the needle reinserted.

It can be noted that while some participants had been infused with a relatively low volume of hypertonic saline (e.g. 0.4 ml, S5, S17), on the other hand some required a comparatively higher amount (e.g. 2.3 ml, S16). There was no significant difference between the total infused volume of hypertonic and isotonic saline ($p = 0.81$).

2.3.2 Perceived Pain Intensity

Infusion of hypertonic saline (0.5%) was associated with moderate pain intensity (3-6 cm on a 10 cm scale) without any reported undesirable side effects during and after the infusion. All participants were able to finish the required jaw movement tasks during the hypertonic saline infusion. The means and standard deviations of the VAS scores rated by each participant during hypertonic saline infusion after the completion of the different jaw tasks are illustrated in table III-5.

Repeated measures Analysis of Variance (ANOVA) of the VAS pain intensity scores after each trial of a jaw task during the hypertonic saline infusion showed no significant effect of repeating the trial (3 trials of ramp and 5 trials of step task) during the ramp tasks ($p = 0.73$) and the step tasks ($p = 0.24$).

The highest mean VAS score was recorded from S7 during the ramp and step level recording at 5.5 ± 1.2 cm whereas the lowest mean score was noted by S5 at 2.5 ± 0.8 cm during the recording of the ramp tasks. Results of a paired sample t-test ($p=0.26$) and an ANOVA of within participants revealed no significant differences between hypertonic saline VAS scores obtained during the 2 jaw tasks ($p = 0.64$).

Table III-5 Means and standard deviations (SDs) for VAS scores obtained from each participant after the completion of each jaw task trial during hypertonic saline infusion

Participant	Mean and SD scores (cm)			
	ramp		step	
	Mean	SD	Mean	SD
P1			4.0	0.7
P2			2.8	1.1
P3			3.0	0.9
P4	4.2	0.3	3.3	0.3
P5	2.5	0.8	4.0	0.9
P6	4.4	0.5	4.3	1.0
P7	5.5	0.8	5.5	1.2
P8	3.1	0.6	5.2	0.4
P9	5.1	1.5	3.3	1.2
P10	4.2	0.3	4.5	0.2
P11	4.2	0.4	4.9	0.6
P12	3.3	0.1	3.0	0.6
P13	3.6	1.0	4.8	0.9
P14	3.2	0.8	4.7	0.4
P15	5.0	0.4	4.0	0.7
P16	3.2	0.2	3.8	0.8
P17	5.0	0.5	4.7	0.5
Mean and SD	4.0	0.4	4.1	0.8

Table III-6 shows the means and standard deviations (SDs) of the VAS scores that were rated by the participants after each jaw task during the infusion of isotonic saline. The highest VAS score was recorded from P4 during the ramp level recording at 2.6 ± 2.1 cm whereas the lowest score was ranked 0 and this score was given by most of the participants.

Table III-6 VAS scores from all participants during isotonic saline infusion

Participant	Mean and SD scores cm			
	Isotonic Ramp		Isotonic Step level	
	Mean	SD	Mean	SD
P1			0.6	0.2
P2			0.2	0.1
P3			0.3	0.1
P4	2.6	2.1	0.1	0.1
P5	1.5	0.6	1.4	0.2
P6	No Data	No Data	No Data	No Data
P7	0.0	0.0	0.0	0.0
P8	0.2	0.3	0.0	0.0
P9	0.4	0.0	0.4	0.1
P10	1.9	0.4	1.2	0.6
P11	0.1	0.1	0.3	0.2
P12	1.5	0.4	1.4	0.1
P13	0.0	0.1	0.0	0.0
P14	0.1	0.1	0.1	0.1
P15	0.0	0.0	0.0	0.0
P16	1.0	0.5	1.2	0.6
P17	0.0	0.0	0	0.0
Mean and SD	0.7	0.6	0.4	0.2

No data = Infusion had not been finished in this participant due to needle instability and the unwillingness of participant to have the needle reinserted.

Repeated measures Analysis of Variance (ANOVA) of the VAS pain intensity scores after each trial of a jaw task during the isotonic saline infusion showed no significant effect of repeating the trial (3 trials of ramp and 5 trials of step task) during the ramp tasks ($p = 0.64$) and the step tasks ($p = 0.62$). On the basis of this result, the mean of the repeated tasks (3 tasks for ramp and 5 tasks for step, see methods) was used in the following analyses.

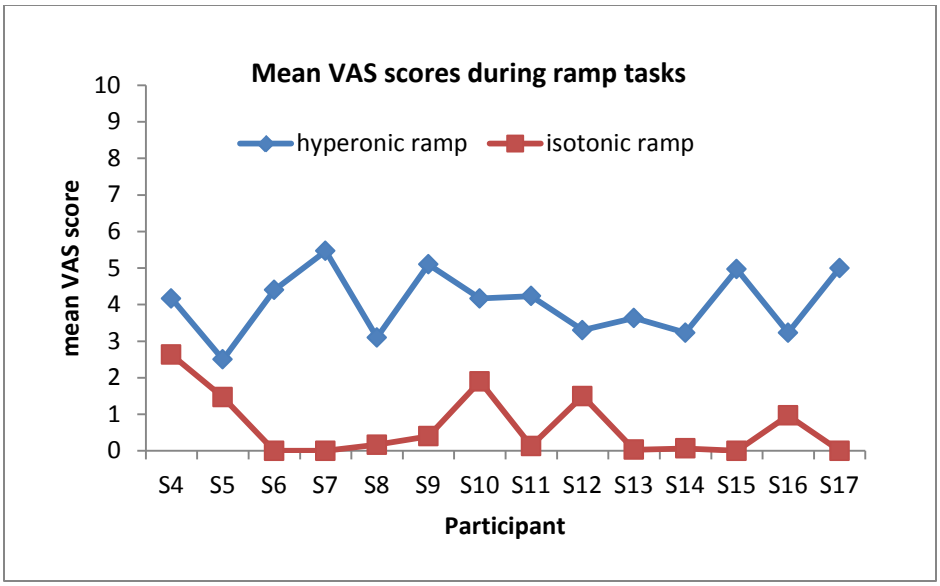


Figure III-2 Mean VAS scores for all participants during hypertonic and isotonic saline infusion after each participant had performed all trials of each ramp task. Most participants performed 3 ramp tasks; 9 some participants performed 1 or 2 extra trials when there was some issue with the recording. e.g. P13 could not follow the target in 2 trials of the ramp and 2 extra trials were recorded to get the appropriate data. However, only 3 ramp trials (the best of 5) were used for analysis.

The analyses of the pain intensity rating between hypertonic saline (4.0± 0.9) in comparison with isotonic saline (0.7±0.9) infusion during the ramp tasks (Figure III-2) demonstrated a statistically significant difference ($p = 0.00$). Paired sample t-test was done to see gender difference in VAS scores of 2 step biting tasks (ramp and step level task) of hypertonic and isotonic infusion sessions. There were no significant differences between males and females for the hypertonic ramp ($p=0.12$), hypertonic step ($p=0.60$), isotonic ramp ($p=0.75$), isotonic step ($p=0.16$).

Paired analyses indicated that there was a statistically significant difference (p = 0.00) in the rating of pain intensity during the hypertonic saline (4.1±0.8) in comparison with the isotonic saline infusion (0.4±0.5) during the step tasks (Figure III-3).

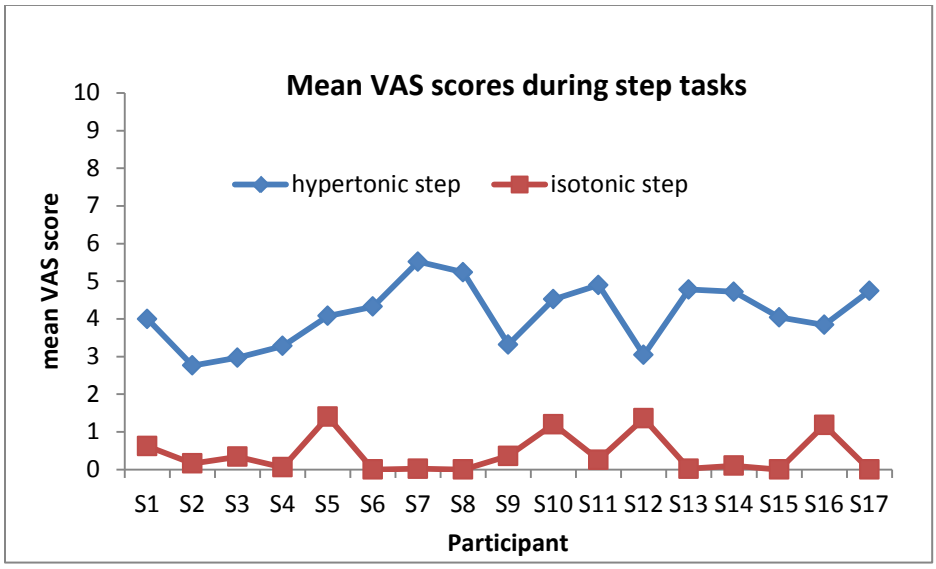


Figure III-3 Mean VAS scores for all participants during hypertonic and isotonic saline infusion after each participant performed each of the 5 trials of each step task. Most participants performed 5-6 step tasks. 1 to 2 extra trials were recorded in the participants if the recorded trial was not satisfactory and in order to get best 5 trials which we used for analysis.

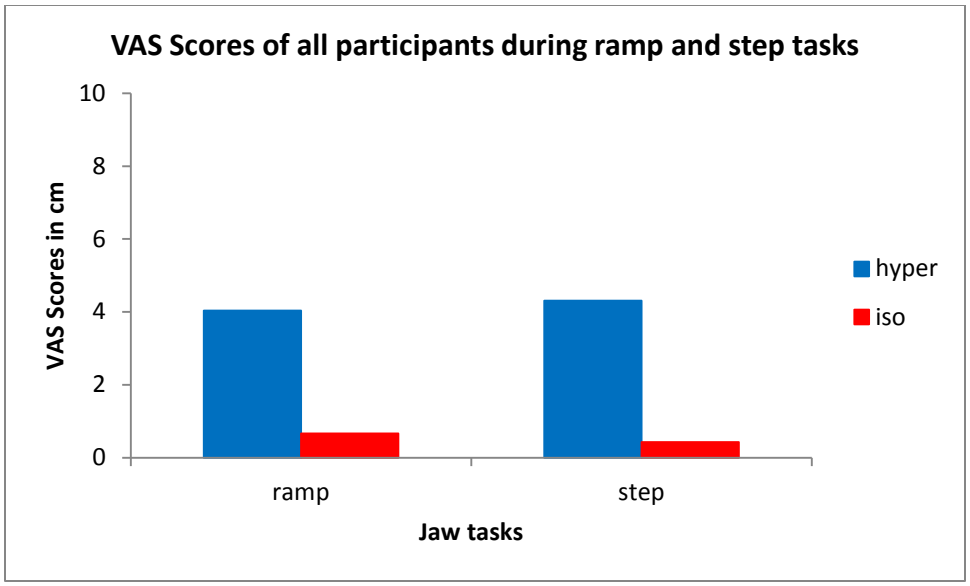


Figure III-4 Visual analogue scale scores of all participants during hypertonic saline infusion (hyper) and isotonic saline infusion (iso) estimated after each participant performed each trial of each jaw task (mean values).

Fig III-4 and III-5 indicates the comparison of mean and standard deviation (SD) score values of Visual analogue scale scores of all participants during hypertonic saline infusion and isotonic saline infusion estimated after each participant performed each trial of each jaw task (ramp and step level tasks). Note the higher scores of hypertonic saline compared to isotonic saline.

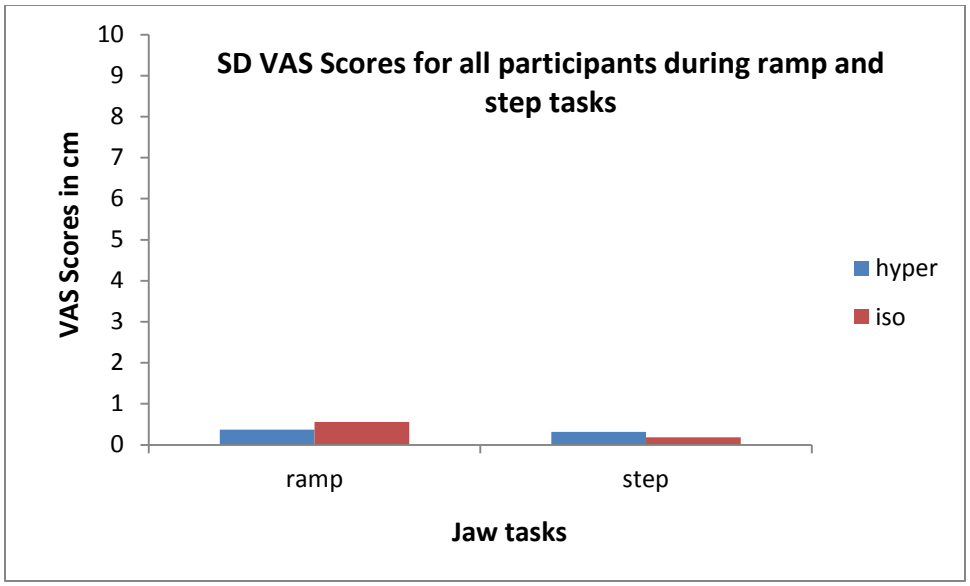


Figure III-5 Visual analogue scale scores of all participants during hypertonic saline infusion and isotonic saline infusion estimated after each participant performed each trial of each jaw task (SD values).

2.3.3 Perceived Pain Areas

After the completion of each jaw task trial during hypertonic saline and isotonic saline infusions, each participant outlined the area of pain experienced on a lateral profile picture of the head and neck. Visual inspection of these indicated that infusion of hypertonic saline into the right masseter muscle produced discrete pain areas originating from the site of the infusion in all participants. In the majority (15/17) of participants, the pain was confined to the boundaries of the infused masseter muscle. Of the 17 participants, 2 reported referral of pain to a remote site during infusion of hypertonic saline. In these 2 participants, pain spread to adjacent ipsilateral sites *i.e.*, in and around the angle of the mandible (P6) and to the right temporalis muscle (P9).

On the other hand, the pain areas drawn by participants who reported pain with isotonic saline infusion were very small (P10, P11) and did not cross the boundaries of the infused masseter muscles. Some of the participants who received isotonic saline injections first reported pain on maps during the initial trials of a task but the extent of these pain areas reduced in size towards the end of the infusion period. None of the participants reported referral of pain to a remote site during isotonic saline infusion.

Table III-7 Number of participants and the location of their pain areas during hypertonic saline (HS) and isotonic saline (IS) infusions.

Participant	Gender	HS	IS	Referred pain	
S1	F	Masseter	no pain	No	
S2	F	Masseter	no pain	No	
S3	F	Masseter	no pain	No	
S4	F	Masseter	no pain	No	
S5	M	Masseter	no pain	No	
S6	M	Masseter	no pain	Angle of mandible	
S7	F	Masseter	no pain	No	
S8	M	Masseter	no pain	No	
S9	M	Masseter	no pain	Temporalis	
S10	F	Masseter	Masseter	No	
S11	F	Masseter	Masseter	No	
S12	M	Masseter	no pain	No	
S13	F	Masseter	no pain	No	
S14	F	Masseter	no pain	No	
S15	F	Masseter	no pain	No	
S16	M	Masseter	no pain	No	
S17	F	Masseter	no pain	No	

2.3.4 Description of Experimental Right Masseter Muscle Pain from the McGill Pain Questionnaire

The quality of pain evoked by hypertonic and isotonic saline infusion was described by the use of the McGill Pain Questionnaire. The total number of times a word was chosen by all of the participants, and their total weighted scores, after the termination of the hypertonic and isotonic saline solution infusions, are illustrated in Tables III-8 and III-9, respectively. The weighted scores were obtained from (Melzak, 1975).

Sharp was the most frequent word used by 9/17 (53%) of participants after hypertonic saline infusion and by 4/17 (23.5%) after isotonic saline infusions. More than 5/17 (29.4%) of participants chose the words *pinching* and *hurting* to describe their pain caused by hypertonic saline while *pinching*, *pricking* and *sore* were the second most common words allocated for isotonic saline, at 3/17 (17.7%) each.

Table III-8 and III-9 show the details of each word chosen by each participant to describe pain induced by hypertonic saline (Table III-8) and isotonic saline infusion (Table III-9).

Table III-8 Number of participants who have chosen each word to describe pain induced by hypertonic saline.

Descriptors	Group	Words	Hypertonic n=17	Weighted Value
Sensory	1	Flicking	1	0.69
		Pulsing	2	4.14
		Throbbing	4	11.04
		Pounding	1	4.14
	2	Shooting	3	12.42
	3	Pricking	1	0.93
		Boring	1	1.86
		Drilling	2	5.58
		Stabbing	3	11.16
		Lancinating	1	4.65
	4	Sharp	9	14.31
	5	Pinching	5	4.05
		Pressing	3	4.86
		Crushing	1	4.05
	6	Pulling	1	2.38
		Wrenching	1	3.57
	7	Burning	3	7.68
	8	Tingling	1	0.7
		Stinging	3	8.4
	9	Dull	3	2.16
		Sore	2	2.88
		Hurting	5	10.8
		Aching	3	8.64
	10	Rasping	1	2.85
Affective	11	Tiring	2	3.48
	13	Fearful	2	3.74
		Terrifying	1	5.61
	14	Cruel	1	3.96
Evaluative	16	Annoying	3	3.03
		Miserable	1	3.03
		Intense	3	12.12
Miscellaneous	17	Spreading	4	4.88
		Radiating	2	7.32
		Penetrating	4	14.64
		Piercing	3	14.64
	18	Squeezing	4	13.12
	19	Cool	1	1
		Cold	1	2
	20	Nagging	1	0.69
		Agonizing	1	4.14
		Torturing	1	11.04

Table III-9 Number of participants who have chosen each word to describe pain induced by isotonic saline.

Descriptors	Group	Words	Isotonic n=16	Weighted Value
Sensory	1	Pulsing	1	2.07
		Beating	1	3.45
		Pounding	1	4.14
	2	Shooting	1	4.14
	3	Pricking	3	2.79
		Stabbing	2	7.44
	4	Sharp	4	6.36
	5	Pinching	3	2.43
		Pressing	1	1.62
	6	Pulling	1	2.38
	7	Hot	1	1.28
	8	Stinging	1	2.8
	9	Dull	2	1.44
		Sore	3	4.32
		Hurting	1	2.16
		Aching	1	2.88
	10	Tender	1	0.95
Affective	11	Tiring	1	1.74
	13	Fearful	1	1.87
Evaluative	16	Annoying	1	1.01
		Intense	1	4.04
Miscellaneous	17	Spreading	1	1.22
		Radiating	1	2.44
		Penetrating	1	3.66
		Piercing	1	4.88
	18	Squeezing	1	3.28
	19	Cool	1	1
		Cold	1	2

Table III-10 shows the mean scale score values that were used to quantify each participant's pain produced by the experimental solutions. The data indicate that hypertonic saline infusion had a greater effect on the miscellaneous descriptors from the MPQ as compared to the other dimensions. A qualitative analysis shows difference in the MPQ Total pain rating indices for hypertonic (5.51) and isotonic saline (1.16) infusions for scale scores (See Table III-10).

Table III-10 The mean scale score values for each McGill Pain Questionnaire pain rating index (PRI) during infusion of hypertonic and isotonic saline infusions.

Pain Rating Index	Hypertonic saline (N=17)	Isotonic saline (N=16)
	Scale score/ Weighted score	Scale score/ Weighted score
Sensory	6.25/5.58	1.65/2.59
Affective	2.50/4.2	1.0/1
Evaluative	6.00/6.12	1.0/2.5
Miscellaneous	7.29/8.23	1.0/2.4
Total	5.51/6.03	1.16/2.13

Similarly, the total weighted score value of hypertonic (6.03) was higher as compared to isotonic (2.13).

3. RECORDING OF SINGLE MOTOR UNITS AT TWO DIFFERENT SITES WITHIN THE MASSETER MUSCLE DURING EXPERIMENTAL PAIN.

In the first 3 participants, recording sites of the SMUs was undertaken at anterior and posterior sites in the right masseter. In the remaining 14 participants, SMUs were

recorded from superior and inferior sites (see Methods) in order to record single motor unit activity.

The distance between the two intramuscular EMG recording sites within the right masseter (RMS/RMA and RMI/RMP) ranged between 9-28 mm.

The data from 12 participants was able to be analysed for SMU activity. The data of the remaining 5 participants was not able to be used for SMU analysis and was analysed for RMS. From the data of the 12 participants, a total of 50 SMUs were discriminated from the two sites of the right masseter muscle. Each SMU was allocated an alphabetical identification number either in upper or lower case. Of the 12 participants, only one step level recording was performed in 3 participants whereas in the remaining 9 participants, the recording of two step levels as well as the ramp tasks recording was performed.

3.1 Force Levels during the Step Level Tasks

Seventeen participants took part in this study. Three participants completed the one step level task only whereas 14 participants completed both the ramp and the two step level tasks. Table III-11 shows the mean force levels of all participants in step level tasks during each recording session. The overall mean force was the same during all three recording sessions. The step level 1 force ranged from 2.65-38.46 N (hypertonic saline infusion) and 1.14-37.91 N (isotonic saline infusion). The step level 2 force ranged from 1.51-61.8 N (hypertonic) and 2.73-57.62 N (isotonic) (Table III-11).

Table III-11 Mean force levels (N) of all the participants in two step level tasks

Participant	Baseline		Hypertonic		Isotonic	
	Level 1	**Level 2**	**Level 1**	**Level 2**	**Level 1**	**Level 2**
P1	17.97	NR	16.90	NR	17.30	NR
P2	12.90	NR	15.26	NR	15.39	NR
P3	38.36	NR	38.46	NR	37.91	NR
P4	8.87	17.15	7.99	15.11	7.43	15.23
P5	4.65	13.97	4.52	12.79	5.21	13.25
P6	8.95	20.93	8.54	21.54	8.34	20.32
P7	5.07	27.18	5.26	29.99	5.58	30.43
P8	13.07	32.68	12.40	32.96	11.50	32.67
P9	2.78	10.70	2.65	10.47	3.18	11.70
P10	14.57	24.06	17.51	32.85	15.44	31.25
P11	13.33	30.06	7.20	24.61	10.44	30.22
P12	6.14	29.16	5.65	27.96	6.34	30.44
P13	0.92	3.58	2.81	1.51	1.14	2.73
P14	14.46	27.05	15.51	26.91	12.83	23.99
p15	7.39	11.01	5.65	9.67	5.77	11.15
P16	10.99	26.88	11.42	26.40	10.45	25.06
P17	24.89	54.16	25.33	61.80	24.52	57.62
Mean	**12.08**	**23.47**	**11.94**	**24.54**	**11.69**	**24.00**

NR=Not recorded

In some of the participants, biting force recorded was very low (participants 9, 13), whereas in some participants the biting force recorded was much higher (3, 17). There were no significant differences between baseline, hypertonic saline infusion and isotonic

saline infusion for the stable force levels analysed at step level 1 and step level 2 (ANOVA P>0.05).

Of the 50 SMUs that were discriminated, 26 SMUs were discriminated in the inferior/posterior site of the right masseter (RMI/RMP) and 24 SMUs were identified at the superior/anterior site of the right masseter (RMS/RMA). Of these 50 SMUs 54% (14/26) were discriminated at right masseter inferior/right masseter posterior (RMI/RMP) site only, 75% (18/24) were identified at right masseter superior/right masseter anterior (RMS/RMA) site only and 64% (32/50) were present at both sites (RMI/RMP and RMS/RMA). Tables III-12 and III-13 provide overall accounts of the presence (+) or absence (-) of each of the SMUs across each of the jaw tasks within each of the sessions for the RMI/RMP site. Table III-14 and III-15 provide the same data for the RMS/RMA site.

3.2 Occurrence of All Single Motor Units Identified at the Right Masseter Inferior/Posterior Site (RMI/RMP)

Table III-12 describes the occurrence of 26 SMU identified at the RMI/RMP sites. This table shows the presence of each SMU in each recording session (BL, HS, IS) at the sites. If a SMU was present (in one or more trials),, it was marked as "+" and if a SMU was not present in a particular recording session, but was present in another recording session, it was marked as "–".

Table III-12 shows data for discriminated SMUs at RMI/RMP (right masseter inferior/posterior) sites during three sessions of recording. For subjects 8, 9 and 11 the data of this site were not able to be analysed due to technical problems.

Table III-12 Occurrence of all single motor units identified at right masseter inferior/posterior site (RMI/RMP)

Ptc		Ramp			Step level 1			Step level 2		
		BL	**HS**	**IS**	**BL**	**HS**	**IS**	**BL**	**HS**	**IS**
1	**A**	NR	NR	NR	+	+	+	NR	NR	NR
	B	NR	NR	NR	+	+	+	NR	NR	NR
	C	NR	NR	NR	-	+	-	NR	NR	NR
2	**D**	NR	NR	NR	+	+	+	NR	NR	NR
	E	NR	NR	NR	+	+	+	NR	NR	NR
	F	NR	NR	NR	+	+	+	NR	NR	NR
	G	NR	NR	NR	-	+	-	NR	NR	NR
3	**H**	NR	NR	NR	+	-	+	NR	NR	NR
	I	NR	NR	NR	+	-	+	NR	NR	NR
	J	NR[i]	NR	NR	+	-	+	NR	NR	NR
4	**M**	+	+	+	+	+	+	+	+	+
	N	+	+	+	+	+	+	+	+	+
	O	+	-	+	-	-	+	+	+	+
5	**S**	+	+	+	+	+	-	+	+	-
	T	+	+	+	+	+	-	+	+	-
	U	+	+	+	+	+	+	+	+	+
	V	-	+	+	-	+	+	-	+	+
6	**Z**	+	+	-	+	+	+	+	+	+
	a	+	+	+	+	+	+	+	+	+
	b	-	-	+	+	+	+	+	+	+
7	**h**	+	+	+	+	+	+	+	+	+
10	**n**	+	+	+	+	+	+	+	+	+
	o	+	+	+	+	+	+	+	+	+
12	**t**	+	+	+	+	+	+	+	+	+
	u	+	+	+	+	-	+	+	+	+
	v	-	+	+	-	-	-	-	+	+
Total	**26**	**13/26**	**14/26**	**15/26**	**21/26**	**20/26**	**21/26**	**14/26**	**16/26**	**14/26**

NR=Not recorded,
Ptc=Participant

There were 26 SMUs that were able to be discriminated at the right masseter inferior/posterior site (see Table III-12). In three participants (1, 2, 3), only one step level recording was done and in total, 10 SMUs were discriminated in these participants at this site. In these 10 SMUs, 5 were present in all three sessions “A, B, D, E, F”, 2 SMUs “C, G” were recruited during hypertonic saline infusion and were not discriminated during the baseline and isotonic infusion sessions, whereas 3 SMUs “H, I, J” were present in the baseline and isotonic sessions but were not discriminated during the hypertonic session of recording.

In the remaining 9 participants, the recording of SMU activity was carried out during step level task as well as in ramp task. In 3 participants (8, 9, 11) the data of this site were not able to be analysed due to technical issues. In 6 participants, 16 SMUs were discriminated in both tasks. Of these 16 SMUs, 11 “M, N, O, U, a, b, h, n, o, t, u”, showed no change in recruitment pattern and were present in all three recording sessions for both tasks. Of the remaining 16 SMUs, 3 “S, T, Z” were present in baseline and hypertonic sessions but were not discriminated during the isotonic session for step 1 and step 2 “units S and T”, or for the ramp “unit Z”. 2 SMUs “V, v” were not recorded in the baseline session but were present in hypertonic and isotonic sessions of ramp and step 2 but not step 1 “v”.

Changes in the recruitment pattern of SMUs for this site were identified in 4 participants (1, 2, 5, and 12). In participant 1, 2 SMUs “A and B” were identified in all three sessions of recording but a new SMU “C” was identified only during the hypertonic saline infusion session. A similar kind of recruitment pattern was seen in participant 2 where 3 SMUs “D, E, and F” were present in all three conditions but a new SMU “G” was

identified in the hypertonic saline infusion session only and was not identified during baseline and isotonic saline infusion sessions. In participant 5, 3 SMUs “S, T and U” were present in all three sessions whereas SMU “V” was not present in the baseline session of the ramp and the Step tasks but was identified in both infusion sessions. Similarly, in participant 12, two SMUs “t” and “u” were identified during all three recording sessions but a new SMU “v” was identified in the two infusion sessions at both ramp and step 2 tasks but was not identified in all tasks of the first session of recording (baseline).

In conclusion, 16 SMUs showed no change in recruitment pattern at RMI/RMP (right masseter inferior/posterior) sites during three sessions of recording but 10 SMUs showed a change in recruitment pattern during one or both infusions at this recording site of masseter.

Table III-13 lists the frequency of occurrence of each single motor unit recorded in each task in each participant under baseline, hypertonic saline and isotonic saline infusion sessions at right masseter inferior/posterior (RMI/RMP) site. In each cell, the denominator refers to the total number of trials carried out, and the numerator refers to the number of trials in which that SMU was identified. A unit need not necessarily be present in every trial of a task in order to be considered to be active in that task in Table III-13. Most of the SMUs were present in most of the trials of all three sessions of recordings (see table III-13). Some SMUs were identified in some sessions of recording and were not identified in another session. For example, SMU “C” was not identified in any of the trials of baseline session (0/6) and was identified in two trials of hypertonic

infusion session (2/5) and again was not identified in any of the trials (0/5) in isotonic infusion session.

Table III-13 Occurrence of single motor units in all trials of recordings at the right masseter inferior/posterior site (RMI/RMP) for the step level task.

Participant	Baseline				Hypertonic saline				Isotonic saline			
	Level 1		Level 2		Level 1		Level 2		Level 1		Level 2	
1.	**A**	6/6	NR	NR	A	5/5	NR	NR	A	4/5	NR	NR
	B	5/6	NR	NR	B	5/5	NR	NR	B	4/5	NR	NR
	C	0/6	NR	NR	C	2/5	NR	NR	C	0/5	NR	NR
2.	**D**	6/6	NR	NR	D	8/8	NR	NR	D	6/6	NR	NR
	E	6/6	NR	NR	E	8/8	NR	NR	E	6/6	NR	NR
	F	6/6	NR	NR	F	8/8	NR	NR	F	6/6	NR	NR
	G	0/6	NR	NR	G	2/8	NR	NR	G	0/6	NR	NR
3.	**H**	7/7	NR	NR	-	-	NR	NR	H	5/6	NR	NR
	I	6/7	NR	NR	-	-	NR	NR	I	4/6	NR	NR
	J	7/7	NR	NR	-	-	NR	NR	J	5/6	NR	NR
4.	**M**	2/5	M	2/5	M	4/5	M	4/5	M	5/5	M	5/5
	N	4/5	N	4/5	N	3/5	N	3/5	N	4/5	N	5/5
	O	0/5	O	4/5	O	0/5	O	4/5	O	1/5	O	5/5
5.	**S**	5/6	S	5/6		4/6	S	4/6	S	0/6	S	0/6
	T	5/6	T	5/6	T	5/6	T	5/6	T	0/6	T	0/6
	U	5/6	U	5/6	U	4/6	U	6/6	U	6/6	U	6/6
	V	0/6	V	0/5	V	4/6	V	4/6	V	5/6	V	5/6
6.	**Z**	4/5	Z	4/5	Z	4/5	Z	4/5	Z	3/5	Z	3/5
	a	3/5	a	3/5	a	4/5	a	4/5	a	3/5	a	3/5
	b	2/5	b	3/5	b	5/5	b	5/5	b	3/5	b	3/5
7.	**h**	5/6	h	5/6	h	5/5	h	5/5	h	6/6	h	6/6
8.	-		-		-		-		-		-	
9.	-		-		-		-		-		-	
10.	**n**	5/5	n	5/5	n	5/5	n	5/5	n	5/5	n	5/5
	o	2/5	o	5/5	o	5/5	o	5/5	o	5/5	o	5/5
11.	-		-		-		-		-		-	
12.	**t**	3/5	t	5/5	t	3/4	t	4/4	t	4/5	t	5/5
	u	1/5	u	5/5	u	0/4	u	4/4	u	1/5	u	5/5
	v	0/5	v	0/5	v	0/4	v	3/4	v	0/5	v	5/5

NR=Not recorded

Similarly, in participant 2, SMU “G” was not identified (0/6) in the first session of recording (baseline) and appears the first time in the hypertonic session of the recording (2/8) and was not present in the isotonic session of the recording (0/6). In participant 5, SMU “V” was not identified in any of the trials of baseline (0/6) and was identified in both the hypertonic infusion session (4/6) and the isotonic infusion session (5/6). Similarly in participant 12, SMU “v” was not identified in any of the trials of the first session of recording (baseline 0/5) and in the first step recording of both the hypertonic and isotonic infusion session (0/4) but was identified in the step 2 recording of the hypertonic session (3/4) and in step 2 recording of the isotonic session (5/5).

3.3 Occurrence of All Single Motor Units Identified at the Right Masseter Superior/Anterior Site (RMS/RMA)

Table III-14 summarizes all discriminated SMUs at the RMS/RMA sites during the three sessions of recording. A total of 24 SMUs was identified from the 10 participants at whom recordings were made at this site. The data of 2 participants (1, 2) was not suitable to analyse at this site due to some technical issues. Of the 24 SMUs, 19 were present in all three sessions of recordings during both ramp and step level tasks. SMU “a” was not discriminated in all three tasks of baseline (ramp, step 1 and 2) and step 1 of isotonic recording sessions but was identified in both the hypertonic saline session (ramp, step 1 and 2) and in the isotonic session of ramp and step 2 tasks. 3 SMUs “W, X, Y” were discriminated in all three tasks (ramp, step 1 and 2) of the baseline trials only and were not identified in any of the tasks of hypertonic and isotonic sessions, whereas SMU “s”

was present in all three tasks of both the hypertonic and isotonic sessions but was not discriminated for ramp and step 1 tasks of the first recording session (baseline).

Table III-14 Occurrence of all single motor units identified at right masseter superior/anterior site (RMS/RMA)

Ptc	SMU	Ramp			Step level 1			Step level 2		
		BL	HS	IS	BL	HS	IS	BL	HS	IS
3	K	NR	NR	NR	+	+	+	NR	NR	NR
	L	NR	NR	NR	+	+	+	NR	NR	NR
4	N	+	+	+	+	+	+	+	+	+
	P	+	+	+	+	+	+	+	+	+
	Q	+	+	+	+	+	+	+	+	+
	R	+	+	+	+	+	+	+	+	+
5	W	+	-	-	+	-	-	+	-	-
	X	+	-	-	+	-	-	+	-	-
	Y	+	-	-	+	-	-	+	-	-
6	c	+	+	+	+	+	+	+	+	+
	d	+	+	+	-	+	+	+	+	+
	e	+	+	+	+	+	+	+	+	+
	a	-	+	+	-	+	-	-	+	+
7	f	+	+	+	+	+	+	+	+	+
	g	+	+	+	+	+	+	+	+	+
8	i	+	+	+	+	+	+	+	+	+
	j	+	+	+	+	+	+	+	+	+
9	k	+	+	+	+	+	+	+	+	+
	l	+	+	+	+	+	+	+	+	+
10	m	+	+	+	+	-	+	+	+	+
11	p	+	+	+	+	+	+	+	+	+
	q	+	+	+	+	-	+	+	+	+
12	r	+	+	+	+	+	+	+	+	+
	s	-	+	+	-	+	+	+	+	+
Total	24	20/24	19/24	19/24	21/24	19/24	20/24	21/24	19/24	19/24

NR=Not recorded,
Ptc=Participant

During the step 1 task in which all 10 participants contributed, out of these 24 SMUs, 21 were present in the first baseline session of recording, 19 SMUs were identified during the hypertonic saline infusion session and 20 SMUs were identified during the isotonic infusion session. During the step 2 task, the data of 9 participants shows that 21 SMUs were discriminated from the baseline session whereas 19 SMUs were identified during both hypertonic and isotonic recording sessions. A change in recruitment pattern was recorded in the data of two participants (participant 6 and 12) at this site.

In participant 6, two SMUs “c, e” were identified in all three recording sessions during each of the jaw tasks and SMU “d” was identified in all three tasks for both hypertonic and isotonic sessions and in the ramp and step level tasks of the baseline session but was not identified in the step 1 task of the baseline session. In comparison, SMU “a” was identified in the hypertonic saline infusion session for both jaw tasks (ramp and step level tasks) and in the isotonic saline infusion for ramp and step level 2 but not the step level 1 of the isotonic session. SMU “a” was not present in the baseline recordings of either of the jaw tasks.

Table III-15 shows the frequency of occurrence of each single motor unit recorded at right masseter superior/anterior site in each task in each participant under baseline, hypertonic saline and isotonic saline infusion sessions. In each cell, the denominator refers to the total number of trials carried out, and the numerator refers to the number of trials in which that SMU was identified. A unit need not necessarily be present in every trial of a task in order to be considered to be active in that task for table III-15.

Table III-15 Number of appearance of single motor units in all trials of three conditions of recordings at right masseter superior/anterior site (RMS/RMA).

Participant	Baseline				Hypertonic saline				Isotonic saline			
	Level 1		Level 2		Level 1		Level 2		Level 1		Level 2	
1.	-		-		-		-		-		-	
2.	-		-		-		-		-		-	
3.	**K**	2/7	NR	NR	K	7/7	NR	NR	K	6/6	NR	NR
	L	4/7	NR	NR	L	7/7	NR	NR	L	6/6	NR	NR
4.	**N**	2/5	N	3/5	N	3/5	N	5/5	N	2/5	N	4/5
	P	3/5	P	5/5	P	1/5	P	5/5	P	5/5	P	5/5
	Q	3/5	Q	5/5	Q	4/5	Q	5/5	Q	4/5	Q	5/5
	R	1/5	R	4/5	R	2/5	R	5/5	R	2/5	R	1/5
5.	**W**	6/6	W	6/6	-	-	-	-	-	-	-	-
	X	0/6	X	6/6	-	-	-	-	-	-	-	-
	Y	0/6	Y	6/6	-		-	-	-	-	-	-
6.	**c**	3/4	c	3/4	c	4/5	c	4/5	c	5/5	c	5/5
	d	0/4	d	3/4	d	1/5	d	4/5	d	2/5	d	4/5
	e	4/4	e	0/4	e	5/5	e	5/5	e	5/5	e	5/5
	a	0/4	a	0/4	a	0/5	a	3/5	a	0/5	a	3/5
7.	**f**	6/6	f	6/6	f	5/5	f	5/5	f	6/6	f	6/6
	g	6/6	g	6/6	g	5/5	g	5/5	g	6/6	g	6/6
8.	**i**	5/5	i	5/5	i	5/5	i	5/5	i	4/5	i	4/5
	j	1/5	j	5/5	j	3/5	j	5/5	j	1/5	j	4/5
9.	**k**	5/6	k	6/6	k	3/5	k	3/5	k	5/5	k	5/5
	l	2/6	l	6/6	l	1/5	l	3/5	l	1/5	l	3/5
10.	**m**	1/5	m	5/5	m	0/5	m	5/5	m	3/5	m	5/5
11.	**p**	4/5	p	5/5	p	3/5	p	5/5	p	5/5	p	5/5
	q	1/5	q	2/5	q	0/5	q	5/5	q	1/5	q	5/5
12.	**r**	4/5	r	5/5	r	4/5	r	4/5	r	5/5	r	5/5
	s	0/5	s	5/5	s	2/5	s	4/5	s	1/5	s	5/5

NR=Not recorded

In participant 6, SMU “d” was not identified in any of the trials of the step level 1 tasks of baseline and first appeared in hypertonic session in one of the trials of step level 1 (1/5) tasks and stayed active in the rest of the tasks of all of the sessions of recordings (baseline, hypertonic and isotonic). On the other hand SMU “a” in the same participant

showed both recruitment and de-recruitment. It was not active initially in any of the trials of baseline and in step level 1 of the hypertonic session and it appeared for the first time in step level 2 of the hypertonic session (3/5) and then became inactive (de-recruited) in step level 1 of the isotonic session but became active again in step level 2 of the isotonic recording session.

In participant 12, SMU “r” was identified in all three sessions across both jaw tasks and a new SMU “s” was identified during all three tasks of the two infusion sessions but was not identified in the ramp and step level 1 tasks of the baseline session and appeared again in step level 2 task of baseline recording session.

3.4 Differences in Recruitment at Two Different Sites of Masseter

The data from the above two tables (Table III-12 and III-14) show that 7 SMUs were newly recruited at the two sites within the masseter during one or both infusion sessions in the ramp task and/or step level task.

Table III-16 shows the details of these newly recruited single motor units at the two sites of the right masseter muscle during the ramp task and the step level task. All 7 SMUs were identified in hypertonic recording sessions during most of the jaw tasks (ramp or step).

Table III-16 rRecruitment of new single motor units at two sites of the right masseter during the ramp and the step level tasks.

SMU	RMS/RMA			RMS/RMA			RMS/RMA			RMI/RMP			RMI/RMP			RMI/RMP		
	Ramp			Step 1			Step 2			Ramp			Step 1			Step 2		
	B	H	IS	B	H	IS	B	H	IS	B	HS	IS	B	H	IS	B	H	IS
C													-	+	-			
G													-	+	-			
V										-	+	+	-	+	+	-	+	+
a	-	+	+	-	+	-	-	+	+									
b										-	-	+	+	+	+	+	+	+
s	-	+	+	-	+	+	+	+	+									
v										-	+	+	-	-	-	-	+	+

RMS/RMA= Right masseter superior/right masseter anterior,
RMI/RMP= right masseter inferior/right masseter posterior
BL= Baseline, HS= Hypertonic saline, IS= Isotonic saline

Table III-16 shows, that of these 7 SMUs, 5 SMUs “C, G, V, b, v” were identified at the right masseter inferior/posterior (RMI/RMP) site during either the ramp or step level tasks, whereas 2 SMUs “a, s” were identified in either the ramp or step level tasks at the right masseter superior/anterior (RMS/RMA) site. Of these 7 SMUs, 5 SMUs “V, v, a, b, s” were present in the isotonic session during all of the tasks performed and 3 of these 5 SMUs “b, V, v” were present at right masseter inferior/posterior (RMI/RMP) site and 2 “a, s” were identified at right masseter superior/anterior (RMS/RMA) site. No SMU was present in the baseline session of the recordings during any task (ramp or step level) except SMUs “s and b” which were identified in step level 2 of the baseline only. As these 2 SMUS “s, b” were not active initially in the ramp and step level tasks of the baseline session and appeared for the first time in the hypertonic “s” and isotonic “b” recording sessions, these are considered as newly recruited SMUs which stayed active at later stages of recordings of all sessions (step level 2).

As 6 of the 7 newly recruited SMUs were present in the hypertonic recording sessions and 5 of the 7 newly recruited SMUs were also present in one or more of the tasks of the isotonic saline infusion sessions, we examined the order of infusion in these 6 participants. In all these units hypertonic saline was the first infusion. These findings suggest that there may be a carryover or order effect keeping these units (5) active during the isotonic recording session as well.

Table III-17 De-recruitment of single motor units at two different sites of the right masseter during ramp and step level tasks.

SMU	Right			Right			Right			Right			Right			Right		
	Ramp			Step 1			Step 2			Ramp			Step 1			Step 2		
	BL	HS	IS	BL	HS	IS	BL	HS	IS	BL	HS	IS	BL	HS	IS	BL	HS	IS
H													+	-	+			
I													+	-	+			
J													+	-	+			
O										+	-	+	-	-	+	+	+	+
S										+	+	+	+	+	-	+	+	-
T										+	+	+	+	+	-	+	+	-
Z										+	+	-	+	+	+	+	+	+
u										+	+	+	+	-	+	+	+	+
W	+	-	-	+	-	-	+	-	-									
X	+	-	-	+	-	-	+	-	-									
Y	+	-	-	+	-	-	+	-	-									
m	+	+	+	+	-	+	+	+	+									
q	+	+	+	+	-	+	+	+	+									

RMS/RMA= Right masseter superior/right masseter anterior,
RMI/RMP= right masseter inferior/right masseter posterior
BL= Baseline, HS= Hypertonic saline, IS= Isotonic saline

Table III-17 explains in detail the de-recruitment of single motor units at the two sites of the masseter muscle in the ramp biting task and the step level biting task. In total, 13 SMUs showed de-recruitment out of which 8 “H, I, J, O, S, T, Z, u” were present at right

masseter inferior/posterior (RMI/RMP) site whereas 5 “W, X, Y, m, q” were identified at right masseter superior/anterior (RMS/RMA) site. Most of these units were present in the first session of recording (baseline) and become inactive in later stages of either hypertonic or isotonic recording sessions in the trials of one or more of the jaw tasks (ramp, step level task).

3.5 Comparison of Occurrence of SMUs at Two Recording Sites within the Masseter Muscle

An analysis was done to see if there was any difference in the pattern of change in occurrence of SMUs at the two sites within the masseter. Comparisons were made between baseline vs. hypertonic and hypertonic vs. isotonic for all three jaw tasks (ramp, step level task) were performed.

Table III-18 Comparison of the patterns of change in occurrence of SMUs between baseline and hypertonic saline infusion during each task. No change = SMUs present under baseline (BL) session and hypertonic saline (HS) session in that task at that site; De-recruit = number of SMUs de-recruited during HS session in comparison with BL session. Recruit = number of SMUs recruited during HS session in comparison with BL session.

	BL vs. HS Ramp		**BL vs. HS Step 1**		**BL vs. HS Step 2**	
	RMS/RMA	**RMI/RMP**	**RMS/RMA**	**RMI/RMP**	**RMS/RMA**	**RMI/RMP**
No Change	17	12	16	19	18	14
De-recruit	3	2	5	4	3	0
Recruit	2	2	3	3	1	2
	5/17	4/12	8/16	7/19	4/18	2/14

Table III-18 summarizes the patterns of change in occurrence of SMUs (recruitment or de-recruitment) at the two recording sites within the right masseter between baseline and hypertonic saline infusion sessions during each jaw task. Note that, qualitatively, there were no changes in SMU occurrence for comparisons of baseline vs. hypertonic infusion at both sites during all the jaw tasks.

Table III-19 shows a comparison of the patterns of change in occurrence of SMUs between hypertonic saline and isotonic saline infusion sessions during each jaw task. No change = SMUs present under baseline (BL) session and hypertonic saline (HS) session in that task at that site; De-recruit = number of SMUs de-recruited during HS session in comparison with BL session. Recruit = number of SMUs recruited during HS session in comparison with BL session.

	HS vs. IS Ramp		**HS vs. IS Step 1**		**HS vs. IS Step 2**	
	RMS/RMA	**RMI/RMP**	**RMS/RMA**	**RMI/RMP**	**RMS/RMA**	**RMI/RMP**
No Change	22	13	21	17	22	14
De-recruit	0	2	2	5	0	0
Recruit	0	1	1	4	0	2
	0/22	3/13	3/21	9/17	0/22	2/14

Table III-19 summarizes the patterns of change in occurrence of SMUs (i.e. recruitment or de-recruitment) between hypertonic saline and isotonic saline infusion during each task.

There were no changes in the occurrence of SMUs during the ramp and step level tasks between the two sites within the masseter. However, during step 1 there was a suggestion that for the comparison of hypertonic saline vs. isotonic saline infusion, there was

significantly (chi-square; $p<0.05$) more likely to be recruitment or de-recruitment of SMUs in comparison with no change at the RMI/RMP masseter site (9:17), than at the RMS/RMA site (3:21).

The next analysis carried out was a comparison of the patterns of change in occurrence of SMUs between the tasks (ramp and step 1 task and ramp and step 2 task) at each site within the right masseter and these analyses are shown in Tables III-20 and III-21. These analyses were carried out only for comparisons of whether the same or a different pattern of SMU activity existed between the hypertonic saline infusion session and the isotonic saline infusion session.

Table III-20 shows a comparison of the patterns of change in occurrence of SMUs between ramp and step1 task and ramp and step 2 task at the right masseter superior/anterior site (RMS/RMA).

RMS/RMA	**Ramp vs. Step 1**		**Ramp vs. Step 2**	
	Ramp	**Step 1**	**Ramp**	**Step 2**
Same pattern	22	21	22	22
Different pattern	0	3	0	0

Table III-21 shows a comparison of the patterns of change in occurrence of SMUs between ramp and step 1 task and ramp and step 2 task at the right masseter inferior/posterior site (RMI/RMP).

RMI/RMP	Ramp vs. Step 1		Ramp vs. Step 2	
	Ramp	Step 1	Ramp	Step 2
Same pattern	13	17	13	14
Different pattern	3	9	3	2

Analysis of whether the presence or not of a SMU under hypertonic saline infusion and isotonic saline infusion was the same under the ramp task in comparison with the Step 2 for both sites of masseter. This is because of the reason that the ramp biting task and step 2 of the step level task require higher force than the step 1 of the step level task. However, the comparison of ramp and step level 1 task, showed a difference in pattern of occurrence of SMUs at both sites of masseter (RMS/RMA=0:3), (RMI/RMP=3:9). For the RMS/RMA site, most of the SMUs had the same pattern for ramp vs. step 1 (22:21), and all the SMUs had the same pattern for ramp vs. step 2 (22:22) (see table III-19). As shown in Tables III-16, SMU "a" was active in both isotonic and hypertonic sessions in the ramp task and in step 2 task but in the isotonic session, SMU "a" was inactive in the step 1 task. Similarly, SMUs "m" and "q" were active in both isotonic and hypertonic sessions in the ramp task and in step 2 task but not in hypertonic session in the step 1 task (see Table III-17).

For the RMI/RMP site, there were proportionally more SMUs with different patterns for both ramp vs. step, and ramp vs. step 2 compared to RMS/RMA site. SMUs "S" and "T"

were active in the hypertonic but not isotonic sessions in the step 1 and step 2 tasks and were active in both hypertonic and isotonic sessions in the ramp task. SMU “u” was active in both isotonic and hypertonic sessions in the ramp task and in step 2 task but not during the hypertonic session in the step 1 task. SMU “v” was active in both isotonic and hypertonic sessions in the ramp task and in step 2 task but neither during hypertonic nor isotonic sessions in the step 1 task.

3.6 Analysis of SMU Activity in Relation to Vicious Cycle Theory and Pain Adaptation Model (VCT and PAM)

There have been many studies in the literature focusing on two theories explaining the relation between pain and muscle activity. An analysis was therefore performed to determine whether the change in the pattern of occurrence of SMUs at the two different sites within the masseter muscle was consistent with the Vicious Cycle Theory or the Pain Adaptation Model. If the SMU was recruited during the hypertonic saline infusion session in comparison with the isotonic saline session (that is the unit was inactive in the isotonic session), the pattern of occurrence of the SMU was considered to be consistent the Vicious Cycle Theory. On the other hand, if the SMU becomes inactive (that is the unit was de-recruited) during the hypertonic infusion session in comparison with the isotonic session (that is, the unit was active in the isotonic session only), this pattern of occurrence was considered to be consistent with the Pain Adaptation Model. Table III-22 and III-23 give the detailed description of this relationship.

Table III-22 indicates whether the changes in pattern of occurrence of a SMU at the inferior/posterior (RMI/RMP) site during hypertonic saline infusion in comparison with isotonic saline infusion corresponded to the recruitment of a SMU during hypertonic saline infusion (and therefore consistent with the Vicious Cycle Theory, (VCT), or a de-recruitment of SMU activity (consistent with the Pain Adaptation Model, PAM).

Table III-22 Occurrence of SMUs at right masseter inferior/posterior (RMI/RMP) site in relation to the Vicious Cycle Theory (VCT) or the Pain Adaptation Model (PAM).

Inf/Post	**Ramp**		**Step 1**		**Step 2**	
SMU	**VCT**	**PAM**	**VCT**	**PAM**	**VCT**	**PAM**
A	NR	NR			NR	NR
B	NR	NR			NR	NR
C	NR	NR	Y		NR	NR
D	NR	NR			NR	NR
E	NR	NR			NR	NR
F	NR	NR			NR	NR
G	NR	NR	Y		NR	NR
H	NR	NR		Y	NR	NR
I	NR	NR		Y	NR	NR
J	NR	NR		Y	NR	NR
M						
N						
O		Y		Y		
S			Y		Y	
T			Y		Y	
U						
V						
Z	Y					
a						
b		Y				
h						
n						
o						
t						
u				Y		
v						
TOTALS	**1**	**2**	**4**	**5**	**2**	**0**

NR=Not recorded

The above table shows that the pattern observed for many units was not consistent with the PAM in the sense that the unit was still active. During the ramp task of this site, the pattern of occurrence of most SMUs were neither consistent with the Vicious Cycle Theory nor the Pain Adaptation Model.

Table III-23 Occurrence of SMUs at the right masseter superior/anterior (RMS/RMA) site in relation to the Vicious Cycle Theory (VCT) or the Pain Adaptation Model (PAM).

Sup/Ant	**Ramp**		**Step 1**		**Step 2**	
SMU	**VCT**	**PAM**	**VCT**	**PAM**	**VCT**	**PAM**
K	NR	NR			NR	NR
L	NR	NR			NR	NR
N						
P						
Q						
R						
W						
X						
Y						
C						
D						
E						
A			Y			
F						
G						
I						
J						
K						
L						
M				Y		
P						
Q				Y		
R						
S						
TOTALS	**0**	**0**	**1**	**2**	**0**	**0**

NR=Not recorded

The pattern of occurrence of only 1 SMU “Z” was consistent with Vicious Cycle Theory and the pattern of occurrence of 2 SMUs “O, b” was consistent with the Pain Adaptation Model during the ramp biting task at the RMI/RMP site. During the step level 1 tasks, the pattern of occurrence of 4 SMUs “C, G, S, T” was consistent with the Vicious Cycle Theory (VCT), and the pattern of occurrence of 5 SMUs “H, I, J, O, u” was consistent with the Pain Adaptation Model (PAM). In the step level 2 task, the pattern of occurrence of 2 SMUs “S, T” was consistent with Vicious Cycle Theory (VCT) and none was consistent with the Pain Adaptation Model (PAM) (see table ШI-22).

Table ШI-23 indicates whether the changes in pattern of occurrence of a SMU at the superior/anterior (RMS/RMA) site during hypertonic saline infusion in comparison with isotonic saline infusion corresponded to the recruitment of a SMU during hypertonic saline infusion (and therefore consistent with the VCT), a de-recruitment of SMU activity (consistent with the PAM). This table shows that at this RMS/RMA site, very few SMUs exhibited changes in the pattern of occurrence consistent with the VCT or with the PAM. During both ramp and step level 2 tasks, changes in the pattern of occurrence of none of the SMUs were consistent with VCT or PAM and in step level 1 task, only 1 SMU “A” was consistent with VCT and 2 “M, Q” were consistent with PAM.

The data in the above two tables indicate that the pattern of occurrence of most of the SMUs showed neither a relation with the Vicious Cycle Theory nor with the Pain Adaptation Model. Therefore, a further analysis was done to determine whether changes

in occurrence of SMUs at each site within the masseter were consistent with the Vicious Cycle Theory or with the Pain Adaptation Model and the results of this analysis are shown in Table III-24 and III-25.

Table III-24 indicates whether the pattern of occurrence of SMUs at the RMI/RMP site during hypertonic saline infusion in comparison with isotonic saline infusion corresponded to an increase in activity at that site during hypertonic saline infusion (and therefore consistent with the VCT), a decrease in activity (consistent with the PAM), or no change in activity (consistent with neither VCT nor PAM).

Table III-24 shows whether the pattern of occurrence of SMUs at the right masseter inferior/posterior (RMI/RMP) site, for the comparison of hypertonic saline infusion in comparison with isotonic saline infusion, was consistent with the Vicious Cycle Theory (VCT) or the Pain Adaptation Model (PAM) or neither.

RMI/RMP	**RAMP**			**STEP 1**			**STEP 2**		
Ptc	**VCT**	**PAM**	**Neither**	**VCT**	**PAM**	**Neither**	**VCT**	**PAM**	**Neither**
1	NR	NR	NR	Y			NR	NR	NR
2	NR	NR	NR	Y			NR	NR	NR
3	NR	NR	NR		Y		NR	NR	NR
4		Y			Y				Y
5			Y	Y			Y		
6	Y	Y				Y			Y
7			Y			Y			Y
10			Y			Y			Y
12			Y		Y				Y
TOTALS	**1**	**2**	**4**	**3**	**3**	**3**	**1**	**0**	**5**

NR=Not recorded
Ptc=Participant

The table III-24 shows that for the ramp biting task and the step 2 biting task, the pattern of occurrence of SMUs at this site showed no relation with either the VCT or the PAM. For the step 1 task, the pattern of occurrence was consistent with the Vicious Cycle Theory at 3 sites, consistent with the Pain Adaptation Model at 3 sites, and consistent with neither at 3 sites.

Table III-25 shows whether the pattern of occurrence of SMUs at the right masseter inferior/posterior ((RMS/RMA) site, for the comparison of hypertonic saline infusion in comparison with isotonic saline infusion, was consistent with the Vicious Cycle Theory (VCT) or the Pain Adaptation Model (PAM) or neither.

RMS/RMA	**RAMP**			**STEP 1**			**STEP 2**		
Ptc	**VCT**	**PAM**	**Neither**	**VCT**	**PAM**	**Neither**	**VCT**	**PAM**	**Neither**
3	NR	NR	NR			Y	NR	NR	NR
4			Y						Y
5			Y			Y			Y
6			Y	Y					Y
7			Y			Y			Y
8			Y			Y			Y
9			Y			Y			Y
10			Y		Y				Y
11			Y		Y				Y
12			Y			Y			Y
TOTALS	**0**	**0**	**9**	**1**	**2**	**6**	**0**	**0**	**9**

NR=Not recorded

Ptc=Participant

A qualitative comparison of the above two tables show that there is a suggestion, that there are more effects not consistent with either VCT or PAM at the RMS/RMA site (9/9, 6/10, 9/9) in the masseter in comparison with the RMI/RMP site (4/6, 3/9, 5/6).

4. THRESHOLDS OF SINGLE MOTOR UNITS

A Univariate ANOVA of all discriminated SMU threshold values for each jaw task across all of the sessions was performed to determine the main effect of repeating the jaw task and of infusing saline into the right masseter muscle. The analysis was also performed for testing any interactions between the repeated jaw tasks and of the infusion sessions on threshold values.

The results showed no significant effect on the threshold values of repeating the task (3 recordings of the ramp task and 5 recordings of step tasks) during the ramp tasks ($p = 0.11$) and the step tasks ($p = 0.89$). A similar result was found for the main effect of session (i.e. baseline, hypertonic infusion, isotonic infusion) across all threshold values for the ramp tasks ($p = 0.56$) and step tasks ($p = 0.10$). On the basis of this result, the mean of the repeated tasks within a session was used to determine differences in threshold values between the RMI/RMP and RMS/RMA sites.

Fig III-6 provides details of the comparison of the means of the threshold values at the two sites within the masseter muscle for the ramp task during the three recording sessions (baseline, hypertonic, isotonic). There were no significant differences in threshold values between the recording sites for the ramp jaw tasks ($p = 0.28$; mean 14.91 (SD: 2.88) for the RMS/RMA site; mean 9.87 (SD: 3.47) for the RMI/RMP site; Figure III-6).

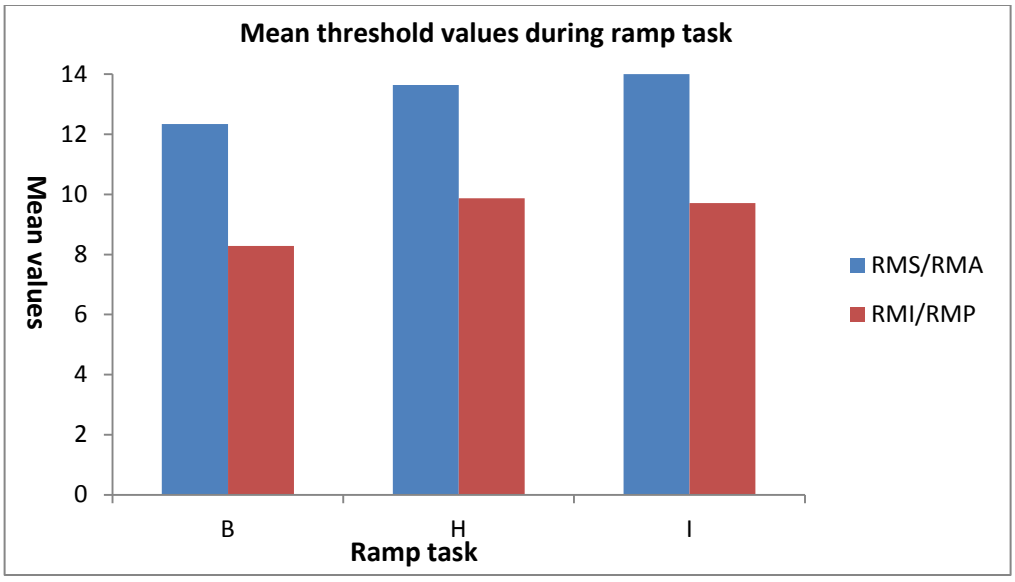


Figure III-6 This figure shows the overall mean threshold values of single motor units recorded during the ramp biting task from the right masseter superior/anterior (RMS/RMA) and inferior/posterior (RMI/RMP) sites across all participants during the three recording sessions (baseline (B), hypertonic (H), isotonic (I)).

Figure III-6 shows the means of the threshold values of two sites (RMS/RMA and RMI/RMP) of masseter during ramp task of all three recording sessions (baseline, hypertonic and isotonic). In analysis of ramp tasks, no significant difference was found in threshold values during the step tasks ($p = 0.11$) between the RMS/RMA site (mean14.2 N, SD 1.8 N), and the RMI/RMP sites (mean 10.0 N, SD 1.9 N).

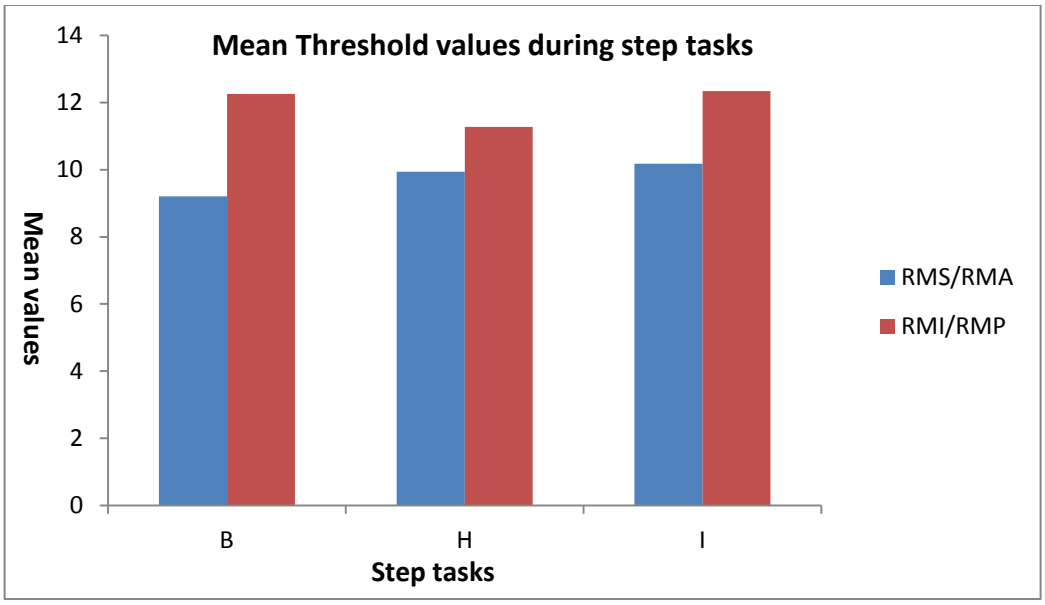


Figure III-7 the figure shows overall means of threshold values of right masseter inferior/posterior (RMI/RMP) and superior/anterior (RMS/RMA) across all participants during three recording sessions (baseline (B), hypertonic (H), isotonic (I)) of step task.

Figure III-7 shows the means of the threshold values of two sites of masseter during step level task of all three recording sessions. Similarly like the ramp analysis, no significant difference was found in threshold values during the step tasks ($p = 0.99$) between the RMS/RMA site (mean 8.9 N, SD 1.8 N), and the RMI/RMP sites (mean 8.9 N, SD 1.9 N).

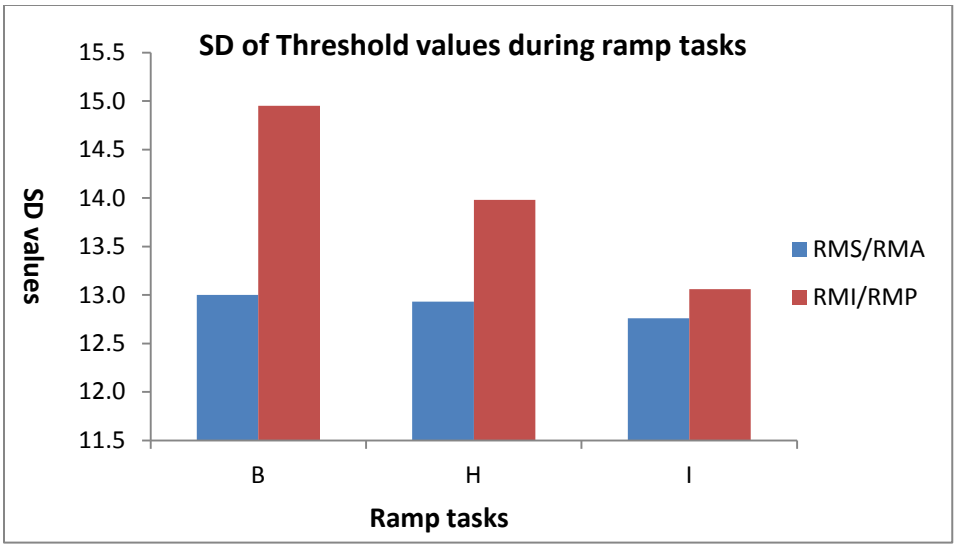


Figure III-8 the figure shows overall standard deviation (SD) of threshold values of right masseter inferior/posterior (RMI/RMP) and superior/anterior (RMS/RMA) across all participants during three recording sessions (baseline (B), hypertonic (H), isotonic (I)) of ramp task.

Figure III-7 & Figure III-8 shows the standard deviation of threshold values of all SMUs of two sites during three recording sessions (baseline, hypertonic, isotonic) of ramp (Figure III-8) and step level (Figure III-9) tasks.

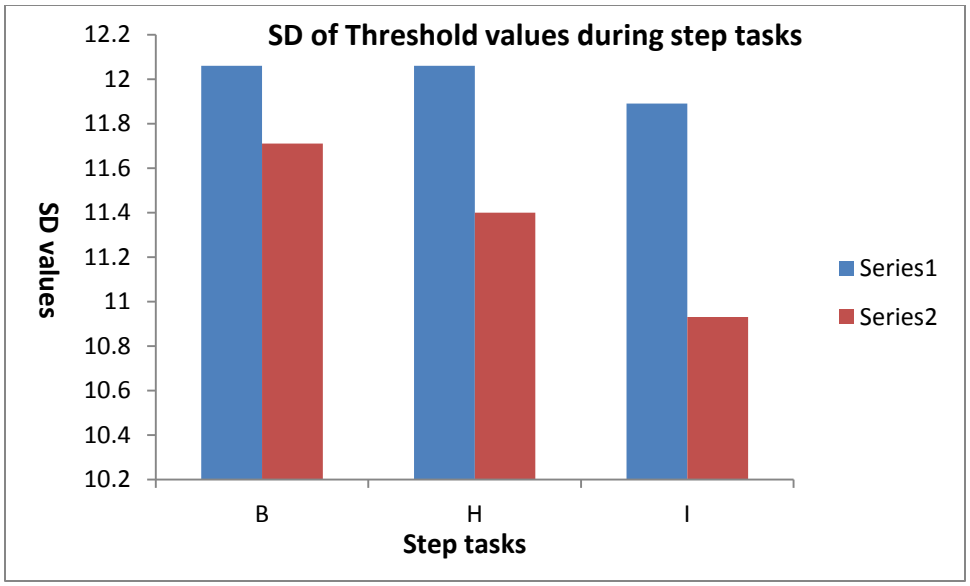


Figure III-9 the figure shows SD of threshold values of right masseter inferior/posterior and superior/anterior across all participants during three recording sessions (baseline, hypertonic, isotonic) of step task.

An analysis was also performed for testing for any interactions between the infusion sessions on threshold values. This was performed to see the effect of hypertonic saline infusion on threshold values. The results showed no significant effect of session across all threshold values for the ramp tasks ($p = 0.56$) and step tasks ($p = 0.10$).

The threshold values (N) of the SMUs for each of the sites discriminated during each session for each of the jaw tasks are shown in table III-26 and table III-27.

Table III-26 Mean threshold values of force (N) for SMUs discriminated for the RMS/RMA site at each recording session for the ramp and the step level biting task. Each value is a mean of 3 trials for the ramp task, and 5 trials for the step level biting task.

Ptc	SMU	Ramp			Step level		
		Baseline	Hypertonic	Isotonic	Baseline	Hypertonic	Isotonic
3.	K	NR	NR	NR	31.65	32.47	30.16
	L	NR	NR	NR	2.35	1.34	1.53
4.	N	3.38	7.85	6.15	2.09	10.61	3.78
	P	2.27	1.51	1.30	1.55	1.52	2.46
	Q	4.76	12.99	14.04	7.45	7.84	9.30
	R	15.32	20.62	20.62	12.61	13.10	9.63
5.	W	3.84	_	_	3.02		
	X	12.40	_	_	8.02		
	Y	9.11	_	_	9.60		
6.	c	21.38	14.03	11.27	9.65	12.27	8.72
	d	21.82	22.36	18.29	10.96	11.22	9.24
	e	2.12	2.63	2.57	23.60	1.91	14.76
	a		2.22	7.83		6.49	6.50
7.	f	2.02	4.26	18.53	2.46	3.12	4.73
	g	2.89	3.88	18.97	2.99	2.52	3.56
8.	i	19.12	32.32	39.09	3.46	6.80	7.14
	j	29.71	30.44	17.60	7.50	10.50	10.83
9.	k	54.70	44.03	54.73	29.86	33.13	21.68
	l	2.64	1.53	3.12	2.44	1.55	2.56
10.	m	7.90	6.42	9.70	9.45	6.67	11.76
11.	p	20.01	17.40	19.41	15.49	13.16	17.26
12.	q	7.27	6.56	1.64	3.15	1.49	1.06
	r	4.24	14.46	8.22	6.90	9.32	9.78
	s		28.52	28.82		21.81	24.77

NR=Not recorded
Ptc=Participant

Table III-27 Mean threshold values in Newton's of force SMUs discriminated for the RMI/RMP site at each recording session for the ramp and the step level biting task. Each value is a mean of 3 trials for the ramp task, and 5 trials for the step level biting task.

Ptc	MU	Ramp			Step level		
		Baseline	Hypertonic	Isotonic	Baseline	Hypertonic	Isotonic
1.	A	NR	NR	NR	3.83	1.93	12.04
	B	NR	NR	NR	1.97	4.27	13.02
	C	NR	NR	NR		0.729	
2.	D	NR	NR	NR	11.89	14.40	9.06
	E	NR	NR	NR	12.11	13.96	9.94
	F	NR	NR	NR	11.89	14.62	13.66
	G	NR	NR	NR		14.31	
3.	H	NR	NR	NR	31.29		32.76
	I	NR	NR	NR	39.45		27.38
	J	NR	NR	NR	36.29		39.34
4.	M	2.06	9.92	2.57	1.64	4.04	4.57
	N	3.38	7.85	6.15	2.09	10.61	3.78
	O	8.30	16.50	12.29	6.37	12.84	9.57
5.	S	1.25	0.87	0.83	1.08	1.03	0.23
	T	1.18	1.03	0.59	1.75	2.12	4.57
	U	2.56	1.47	2.15	2.01	1.03	2.91
	V		0.86	0.84		3.22	4.19
6.	Z	7.41	7.41		3.25	2.84	5.31
	a		2.22	7.83	5.66	6.50	9.24
	b			2.56	7.64	4.41	6.26
7.	h	19.12	32.32	39.09	3.46	6.80	7.14
10.	n	5.84	10.93	10.09	13.11	17.30	10.16
	o	7.15	10.93	13.60	16.27	20.98	17.21
12.	t	4.80	15.77	7.78	7.24	8.54	3.99
	u	29.56	21.03	28.83	24.67	23.77	25.04
	v		22.34	28.39		20.77	23.20

NR=Not recorded
Ptc=Participant

4.1 Sequence of Recruitment of SMUs at Right Masseter Superior/Anterior (RMS/RMA) Site during Ramp and Step Tasks

The data in Table III-26 were further analyzed to see whether the sequence of recruitment of SMUs at a site within the masseter during the ramp and the step task was altered during the infusions.

Table III-28 Sequence of recruitment of single motor units at right masseter superior/anterior (RMS/RMA) site during ramp task. Each SMU is indicated by a letter and the order in which each SMU is recruited is indicated by the sequence of the letters.

Participant	BL	HS	IS	BL vs. HS	HS vs. IS	BL vs. IS
4	PNQR	PNQR	PNQR	Same	Same	Same
6	ecd	aecd	eacd	Different	Same	Different
7	fg	gf	fg	Different	Different	Same
8	ij	ji	ji	Different	Same	Different
9	lk	lk	lk	Same	Same	Same
12	rq	qr	qrs	Different	Same	Different

Table III-28 shows, for the ramp task, the sequence of SMU recruitment calculated from the mean thresholds under baseline (BL), hypertonic saline infusion (HS), and isotonic saline infusion (IS). At each site an assessment is made whether the sequence was altered for BL vs. HS, HS vs. IS and BL vs. IS.

In most of participants, the sequence of SMUs at RMS/RMA site for the ramp task was different between baseline and hypertonic (6, 7, 8, 12) and baseline and isotonic saline

infusions (6, 8, 12) but was the same between hypertonic saline and isotonic saline infusions (4, 6, 8, 9, 12).

Table III-29 shows the same analysis of SMUs of right masseter superior/anterior (RMS/RMA) for the step task

Table III-29 Sequence of recruitment of units at right masseter superior/anterior (RMS/RMA) site during step task. Each SMU is indicated by a letter and the order in which each SMU is recruited is indicated by the sequence of the letters.

Participant	BL	HS	IS	BL vs. HS	HS vs. IS	BL vs. IS
3	LK	LK	LK	Same	Same	Same
4	PNQR	PQNR	PNQR	Different	Different	Same
6	cde	eadc	cde	Different	Different	Same
7	fg	gf	gf	Different	Same	Different
8	ij	ij	ij	Same	Same	Same
9	lk	lk	lk	Same	Same	Same
12	qr	qrs	qrs	Same	Same	Same

Table III-29 shows that the sequence of SMUs was the same in most of the participants comparing baseline and hypertonic saline (3, 8, 9, and 12), hypertonic and isotonic saline (3, 7, 8, 9, and 12) and baseline and isotonic infusion (3, 4, 6, 8, 9, and 12).

An analysis was performed to assess the effect of hypertonic saline infusion on the threshold values. For each SMU during each jaw task, an analysis was carried out as to whether thresholds increased or decreased in each task for comparisons of HS vs. IS infusion. During the ramp task, 10 SMUs exhibited an increase in threshold and 8 exhibited a decrease in threshold during HS in comparison with IS infusion. During the

step task, 7 SMUs exhibited an increase in threshold and 14 exhibited a decrease in threshold during HS in comparison with IS infusion (Table III-26).

4.2 Sequence of Recruitment of SMUs at Right Masseter Inferior/Posterior (RMI/RMP) Site during Ramp and Step Tasks

Similarly**,** the data of Table III-27 was analyzed to determine whether the sequence of recruitment of SMUs at a site within the masseter during the ramp biting task and the step level biting task was altered during the infusions.

Table III-30 Sequence of recruitment of single motor units at right masseter inferior/posterior (RMI/RMP) site during ramp task. Each SMU is indicated by a letter and the order in which each SMU is recruited is indicated by the sequence of the letters.

Participant	BL	HS	IS	BL vs. HS	HS vs. IS	BL vs. IS
4	MNO	MNO	MNO	Same	Same	Same
5	TSU	VSTU	TSVU	Different	Different	Different
6	z	az	ba	Different	Different	Different
10	no	no	no	Same	Same	Same
12	tu	tuv	tvu	Different	Same	Different

Table III-30 shows the sequence of recruitment of SMUs at RMI/RMP site during ramp task. At each site an assessment is made whether the sequence was altered for BL vs. HS, HS vs. IS and BL vs. IS. In most of the participants, sequence of SMUs was different between baseline and hypertonic saline (5, 6, and 12), baseline and isotonic saline (5, 6, and 12) but was the same for hypertonic and isotonic saline (4, 10, and 12).

Table III-31 Sequence of recruitment of single motor units at right masseter inferior/posterior (RMI/RMP) site during step task. Each SMU is indicated by a letter and the order in which each SMU is recruited is indicated by the sequence of the letters.

Participant	BL	HS	IS	BL vs. HS	HS vs. IS	BL vs. IS
1	BA	CAB	AB	Different	Different	Different
2	DFE	EGDF	DEF	Different	Different	Different
4	MNO	MNO	NMO	Different	Different	Same
5	TUVS	SUTV	SUVT	Different	Different	Different
6	zab	zba	zba	Different	Same	Different
10	no	no	no	Same	Same	Same
12	tu	tvu	tvu	Same	Same	Different

Table III-31 shows the sequence of SMUs calculated from the mean thresholds under baseline (BL), hypertonic saline infusion (HS), and isotonic saline infusion (IS) of RMI/RMP during step tasks. In most of the participants, sequence of recruitment was different when we compare baseline vs. hypertonic (1, 2, 4, 5, 6), hypertonic vs. isotonic saline (1, 2, 4, 5) and baseline vs. isotonic saline infusion (1, 2, 5, 6, 12).

For each SMU during each jaw task, an analysis was carried out as to whether thresholds increased or decreased in each task for comparisons of HS vs. IS infusion. During the ramp task, 8 SMUs exhibited an increase in threshold and 7 exhibited a decrease in threshold during HS in comparison with IS infusion. On the other hand, during step level task, 14 SMUs exhibited a decrease and 7 SMUs exhibited an increase in threshold during hypertonic saline in comparison with isotonic saline infusion (Table III-27).

5. ROOT MEAN SQUARE (RMS) VALUES OF RIGHT MASSETER SITES

In 5 of the 17 participants, discrimination of individual SMUs for threshold analysis was not possible. Therefore, to capture the differences in the EMG activity for all of the participants, the data were re-analyzed using the root mean square (RMS) of the EMG signal (see Methods).

Paired sample t-tests were done to determine if there was any effect of repeating the jaw tasks of each session at each site (RMS/RMA and RMI/RMP). There was no significant effect on the root mean square EMG activity of repeating the ramp tasks ($p=0.32$), or step level tasks ($p=0.30$) at the RMS/RMA sites or at the RMI/RMP sites (ramp task, $p=0.42$; step level task, $p=0.52$). Pairwise comparisons (intraindividual), with Bonferroni corrections, showed no significant differences within the ramp tasks ($p=0.79$) and within the step level tasks ($p=0.62$). On the basis of these results, the means of the repeated tasks for each individual (3 tasks for ramp and 5 tasks for step, see Methods) was used in the following analyses.

A mixed analysis was performed to determine whether there were the differences in root mean square masseter EMG activity between each session within a site and between the sites. There was no significant effect of pain across both sites (Greenhouse-Geisser $p=0.52$). Pairwise comparison of the two sites RMS/RMA and RMI/RMP was performed

irrespective of the session and no significant difference was found in the root mean square EMG activity between the sites (p=0.60).

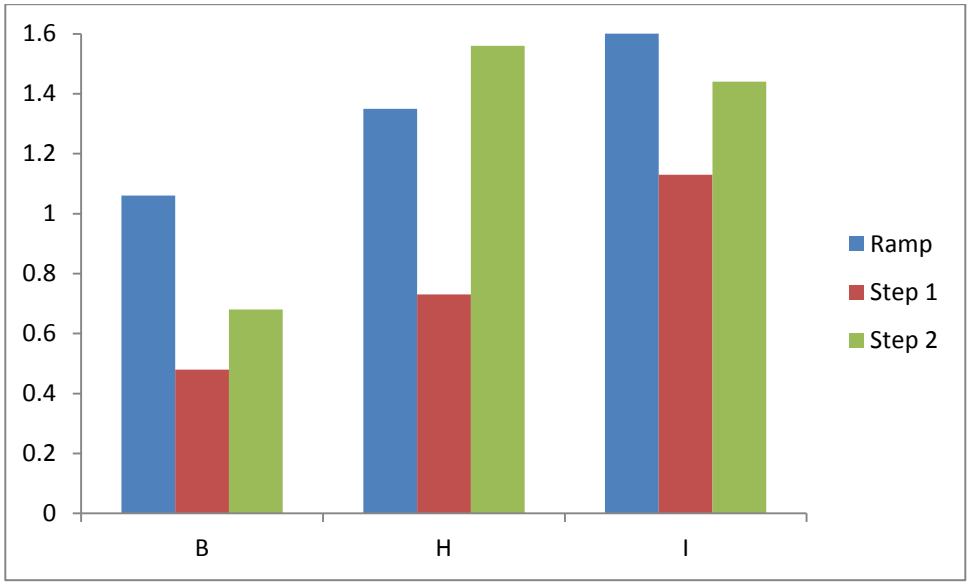


Figure III-10 The overall means of the root mean square (RMS) EMG activity from the right masseter superior/anterior (RMS/RMA) site across all participants during each jaw task (ramp, step 1, step 2) during each recording session (baseline (B), hypertonic (H), isotonic (I)).

The overall means of the root mean square (RMS) EMG activity from the right masseter during each jaw task for each recording session is shown in Figure III-10. During the ramp biting task at this site, the mean RMS values ranged between 1.06-1.67. In the step 1 task the overall mean RMS values ranged 0.48- 1.13 whereas for the step 2 task the mean RMS values ranged between 0.68-1.56.

Table III-32 Root mean square (RMS) EMG activity from the RMS/RMA sites during ramp task, step 1 and step 2 tasks under each recording session.

Ptc	Ramp (n=14)			Step level 1 (n=17)			Step level 2 (n=14)		
	BL	HS	IS	BL	HS	IS	BL	HS	IS
1	NR	NR	NR	0.34	0.26	0.25	NR	NR	NR
2	NR	NR	NR	0.06	0.08	0.05	NR	NR	NR
3	NR	NR	NR	0.18	0.03	0.15	NR	NR	NR
4	0.74	0.23	0.35	0.13	0.61	0.16	0.27	1.16	0.25
5	0.45	0.31	0.36	0.20	0.13	0.14	0.33	0.19	0.22
6	0.69	0.51	0.78	0.34	0.22	0.48	0.58	0.52	0.68
7	4.23	4.34	3.94	0.54	0.83	0.76	1.32	2.43	1.56
8	0.89	1.13	1.00	0.36	0.47	0.54	0.79	1.08	0.91
9	0.08	0.22	0.29	0.18	0.24	0.28	0.25	0.29	0.31
10	0.06	0.09	0.10	0.10	0.07	0.06	0.10	0.10	0.11
11	0.25	0.18	0.16	0.14	0.15	0.11	0.16	0.20	0.18
12	2.38	1.99	2.36	0.80	1.10	0.87	1.54	1.65	1.34
13	1.84	5.94	11.35	1.59	5.43	12.40	1.76	6.24	12.37
14	1.23	0.82	0.44	0.52	0.32	0.31	0.33	0.30	0.36
15	0.23	0.19	0.46	0.19	0.32	0.28	0.32	0.57	0.42
16	0.63	0.67	0.70	0.34	0.32	0.30	0.44	0.46	0.45
17	1.09	2.28	1.09	2.21	1.89	2.05	1.39	6.66	0.95

BL= Baseline, HS= Hypertonic saline, IS= Isotonic saline

Ptc=Participant

Table III-32 show the mean RMS values for each of the participants across each of the sessions for each of the jaw tasks for the RMS/RMA site in the right masseter muscle.

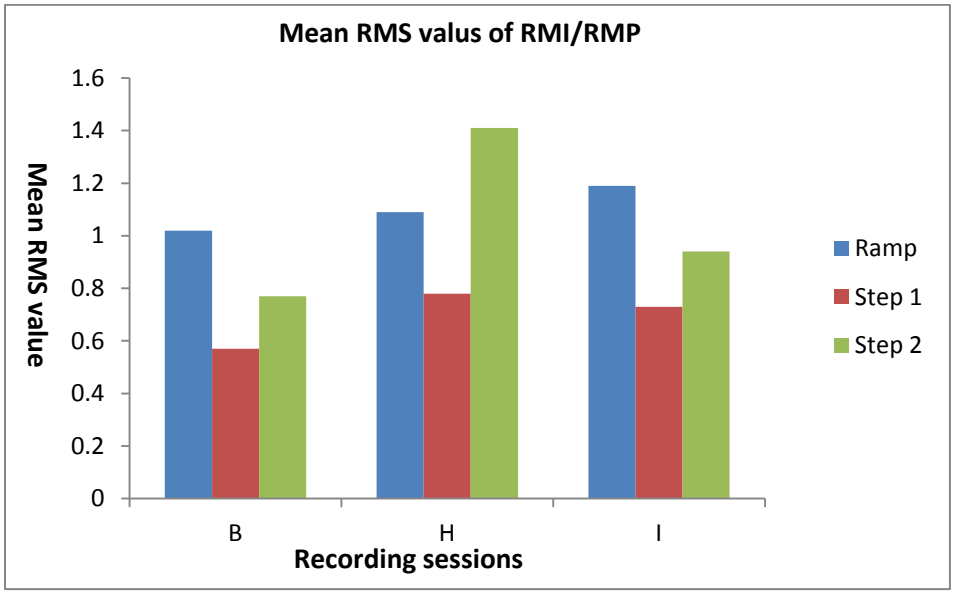


Figure III-11 the figure shows overall means of RMS values of right masseter inferior/posterior (RMI/RMP) across all participants during each recording sessions (baseline, hypertonic, isotonic) of each jaw tasks (ramp, step 1, step 2).

Figure III-11 shows that during the ramp task the mean RMS activity value ranges between 1.02- 1.19 during three recording sessions. In step level 1 task, the value of mean RMS ranges between 0.57-0.78 in all three recording sessions whereas in step level 2 tasks, the mean RMS value ranges between 0.77-1.41.The reason for low values in step level 1 task compared to ramp and step level 2 task is step level 1 task required low force to perform as compared to the other two tasks.

Table III-33 shows the Mean RMS values for each of the participants across each of the sessions for each of the jaw tasks for the RMI/RMP site in the right masseter. The data froms Table III-32 and Table III-33 were further analyzed to count the number of increases and decreases of RMS activity for comparison of hypertonic vs. isotonic infusion sessions at both sites. These data are summarized in Table III-34.

Table III-33 RMS values of right masseter inferior/posterior (RMI/RMP) during ramp, step 1 and step 2 tasks.

Ptc	Ramp (n=14)			Step level 1 (n=17)			Step level 2 (n=14)		
	BL	HS	IS	BL	HS	IS	BL	HS	IS
1	NR	NR	NR	0.15	0.02	0.11	NR	NR	NR
2	NR	NR	NR	0.14	0.15	0.12	NR	NR	NR
3	NR	NR	NR	0.64	0.64	0.63	NR	NR	NR
4	0.74	0.47	1.00	0.21	1.19	0.56	0.44	4.67	0.64
5	0.62	0.59	0.74	0.31	0.51	0.65	0.39	0.51	0.69
6	0.59	1.01	0.75	0.34	0.46	0.76	0.39	0.49	0.75
7	2.93	3.55	3.35	1.44	1.87	1.93	1.65	2.29	2.19
8	0.85	1.29	1.09	1.02	1.21	1.09	1.03	1.24	1.10
9	0.11	0.38	0.33	0.29	0.31	0.37	0.38	0.43	0.48
10	0.23	0.10	0.50	0.11	0.14	0.21	0.14	0.17	0.20
11	0.47	0.99	0.34	0.29	0.53	0.23	0.35	0.63	0.47
12	1.92	2.06	2.73	1.77	1.96	1.93	1.79	2.23	1.85
13	0.90	0.88	1.46	0.34	0.80	1.50	0.63	1.02	1.61
14	2.34	0.09	0.03	0.84	0.09	0.10	1.00	0.07	0.09
15	1.55	2.39	1.52	0.77	1.24	1.07	1.47	2.18	1.76
16	0.45	0.47	0.51	0.12	0.27	0.22	0.19	0.30	0.34
17	0.55	0.97	2.28	0.85	1.90	0.90	0.90	3.53	1.02

NR=Not recorded
BL= Baseline, HS= Hypertonic saline, IS= Isotonic saline
Ptc=Participant

There were no apparent differences between the 2 sites (RMS/RMA and RMI/RMP) in the number of increases and decreases in activity for comparisons of hypertonic vs. isotonic infusion.

Table III-34 Number of increases and decreases of root mean square EMG activity for comparisons of hypertonic vs. isotonic during each jaw task. An increase was defined as occurring if EMG activity in HS was greater than that in IS; a decrease was defined as occurring if EMG activity in HS was less than that in IS.

Site	Change	Ramp (n=14)	Step level 1 (n=17)	Step level 2 (n=14)
RMS/RMA	Decrease	9	7	6
	Increase	5	10	8
RMI/RMP	Decrease	7	9	7
	Increase	7	8	7

6. FIRING RATES OF DISCRIMINATED SINGLE MOTOR UNITS (SMUS)

The data of 50 discriminated SMUs was further analyzed to calculate the firing rates of these discriminated SMUs during the holding phases of the step 1 of the step level biting task. The firing rates of 20 of the 50 SMUs were selected for analysis on the basis that these 20 SMUs were able to be clearly discriminated during the step 1 of the step level biting task so that an unambiguous assessment of the firing rates could be made. An

analysis was done to see if there was any difference in firing rates of discriminated SMUs between hypertonic saline infusion and isotonic saline infusion.

Table III-35 Mean firing rates (action potentials/second) of 20 discriminated SMUs at two sites within the right masseter during hypertonic and isotonic recording sessions.

Ptc	SMU	Firing rate		Mean force level	Mean force level	Site of occurrence
		Hypertonic	Isotonic	Hypertonic	Isotonic	
1	A	16.36	19.54	16.90	17.30	RMI/RMP
2	B	20.32	21.56	16.90	17.30	RMI/RMP
3	D	25.37	23.18	15.26	15.39	RMI/RMP
4	E	18.12	19.43	15.26	15.39	RMI/RMP
5	F	21.74	19.91	15.26	15.39	RMI/RMP
6	K	25.21	20.43	38.46	37.91	RMS/RMA
7	L	14.67	9.52	38.46	37.91	RMS/RMA
8	P	17.11	19.65	7.99	7.43	RMS/RMA
9	Z	11.75	10.13	8.54	8.34	RMS/RMA
10	a	13.68	16.49	8.54	8.34	RMS/RMA
11	b	14.95	15.26	8.54	8.34	RMS/RMA
12	c	13.78	12.72	8.54	8.34	RMI/RMP
13	f	13.51	14.13	5.26	5.58	RMS/RMA
14	g	14.18	15.42	5.26	5.58	RMS/RMA
15	h	21.08	24.01	5.26	5.58	RMI/RMP
16	n	19.46	12.85	17.51	15.44	RMI/RMP
17	o	13.97	8.72	17.51	15.44	RMI/RMP
18	k	13.50	16.30	2.65	3.18	RMS/RMA
19	l	6.98	3.19	2.65	3.18	RMS/RMA
20	i	12.42	14.59	12.40	11.50	RMS/RMA
	Mean	16.40	15.85	13.35	13.14	

Ptc=Participant

Table III-35 shows the mean firing rates (mean of the 5 trials of each session) of 20 discriminated SMUs during hypertonic and isotonic recording sessions. A qualitative analysis was carried out and of these 20 SMUs, 11 single motor units “A, B, E, P, a, b, f,

g, h, k, i" showed an increase and 9 single motor units "D, F, K, L, Z, c, n, o, l" showed a decrease in firing rates when comparing hypertonic vs. isotonic recording sessions.

A qualitative analysis of 2 sites (RMS/RMA and RMI/RMP) was carried out and of these 20 SMUs 9 SMUs was discriminated at right masseter inferior/posterior (RMI/RMP) site and 11 SMUs were discriminated at right masseter superior/anterior (RMS/RMA) site. At RMI/RMP 4 SMUs "A, B, E, h" showed increase and 5 SMUs "D, F, c, n, o" showed an increase in firing rate. At RMS/RMA site 8 SMUs "P, a, b, f, g, h, k, I" showed an increase and only 3 SMUs "K, L, Z" showed decrease in firing rate when comparing hypertonic vs. isotonic recording sessions.

7. THE INFLUENCE OF PSYCHOLOGICAL VARIABLE ON JAW MOTOR ACTIVITY DURING PAIN

A qualitative analysis was done to see the correlation between change in single motor unit characteristics and psychological variables. We looked at the PCS scores of the individuals where occurrence of SMU activity was altered during the hypertonic infusion session compared to the isotonic infusion session. Across both sites, in 5 participants (S1, S2, S11, S16, and S17) there were 6 (12% of total of 50 SMUs) newly recruited single motor units during HS infusion that were not present during IS infusion.These 5 participants (S1, S2, S11, S16, and S17) had a PCS score of (Total PCS=10) which was not different from the PCS score (Total PCS=9.0) of the rest of the participants (S3, S4, S5, S6, S7, S8, S9, S10, S12, S13, S14, S15) where no change in pattern of occurrence of SMU was observed.

CHAPTER IV

DISCUSSION

In the past few decades, several remarkable advancements have been made in terms of understanding pain mechanisms (i.e. recognition of the need to understand the multidimensionality of pain, the biopsychosocial factors involved, and the peripheral and central nociceptive processes involved), diagnosis (e.g. brain imaging and molecular biology) and management (improvements in surgical, pharmacological and behavioral management). Despite these advances, considerable gaps in knowledge still exist in our understanding of pain. One area in which knowledge is lacking is in understanding the effect of pain on motor activity. Such an understanding is important if changes in motor activity in pain could contribute to maintenance or recurrence of symptoms.

There are two main theories explaining the relation between pain and motor activity that have been proposed in the literature: the *Vicious Cycle Theory* (Travell, et al., 1942) and the *Pain Adaptation Model* (Lund, et al., 1991). According to the *Vicious Cycle Theory* a painful muscle becomes hyperactive, irrespective of the task, which leads to ischemia due to vascular compromise and further pain as a result of the accumulation of unwanted chemicals. However, this theory has never gained support from critical reviews of the trigeminal literature (Lund, et al., 1991).

The *Pain Adaptation Model*, on the other hand, proposes change in motor behavior that represents a functional adaptive response that is brought about by inhibition of painful

muscles when they act as agonists and excitation of the antagonist muscles. These changes in muscle activity cause slower and smaller movement which protect the painful muscle from further injury, relieve pain and maintain homeostasis. Although this theory has been supported by many clinical and experimental studies, the *Pain Adaptation Model* does not take into account the multidimensional nature of pain which consists of the sensory-discriminative, cognitive-evaluative, and motivational-affective dimensions (Murray and Peck, 2007) and does not seem to explain the variable range of changes possible in the motor adaptation to pain (Hodges and Tucker, 2011). One of the major problems with these theories is that both incriminate brainstem or spinal Lund, et al., 1991cord reflexes in pain related motor changes and omit the role of psychological factors in this interaction.

More recent theories have been proposed which have attempted to take into account the comprehensive organization of the jaw motor system together with the psychological dimensions of pain in order to understand the effects of pain on muscle activity (Murray & Peck, 2007; Hodges and Tucker, 2011). Recent attempts have been made to test some of these newer theories and human experimental pain models have served an important role in these more recent attempts. However, translating the information from experimental pain studies to the clinical pain condition is problematic given that healthy participants clearly differ from TMD pain patients in term of psychosocial measures such as e.g. catastrophizing, somatization, depression, anxiety and stress (Svensson et al., 2007, Castrillon et al., 2008).

Numerous experimental pain studies have focused on the effects of experimental pain on jaw motor activity in the masseter muscle (e.g. Svensson, et al., 1996; Svensson, et al., 1997; Sae-Lee, et al., 2008a, b; for review Murray and Peck, 2007), and these studies have reported changes in surface EMG activity in association with tasks during pain and the results from some of these studies are not always consistent. However, surface EMG recordings report global levels of activity within a muscle and are unable to reveal any details of changes in motor unit activity within a muscle. A recent single motor unit study has demonstrated that experimental painful stimulation of the masseter muscle results in both increases and decreases in motor unit activity within the muscle during the generation of the same direction and level of force (Minami, et al., 2013). These data suggested a reorganization of motor unit activity occurs within the masseter muscle during pain and provide data consistent with the more recent models (Murray and Peck, 2007; Hodges and Tucker, 2011). However, this study (Minami et al., 2013) has only reported the recruitment and the firing rates of single motor units at a single force level and at the same site within the masseter muscle. It is unclear the general applicability of this reorganization within the masseter, that is, whether this reorganization occurs at a number of different sites within the masseter muscle. It is also unclear as to the general applicability of this reorganization to different motor tasks, for example, whether the changes in single motor unit activity are observed at different force levels, different rates of change of force, and whether there are changes in the threshold of onset of firing of single motor units. Such information would help to clarify the fine details of the effects of pain on muscle activity.

Given the limitation of the information and conflicting findings from the previous studies as mentioned above, the general aim of our study was to determine whether experimental masseter muscle pain resulted in a change in muscle activity at 2 different sites within the masseter muscle during the performance of isometric jaw-closing tasks in asymptomatic participants. To achieve this general aim, a methodology was developed to study the effects of experimental masseter muscle pain induced by hypertonic saline infusion on motor unit activity at 2 different sites within the masseter and during the performance of isometric jaw-closing tasks in asymptomatic participants.

The specific aims of current study were:

1. To determine whether experimental masseter muscle pain alters the ability of individuals to perform isometric jaw-closing tasks.
2. To determine whether experimental masseter muscle pain leads to changes in recruitment patterns, thresholds of firing, or firing rates of single motor units, and changes in root mean square EMG activity, within the masseter muscle during standardized isometric jaw-closing tasks and these changes occur at 2 separate sites within the masseter muscle.
3. To determine whether any changes in recruitment patterns, thresholds of firing, or firing rates of single motor units, or changes in root mean square EMG activity at one site within the masseter muscle during standardized isometric jaw-closing tasks are different to any changes occurring at another site within the muscle.

4. To determine whether experimental masseter muscle pain leads to changes in EMG activity at one or both sites within the masseter that are consistent with earlier theories of pain-motor interaction, namely, the Vicious Cycle Theory and the Pain Adaptation Model.
5. To explore possible associations of pattern of occurrence of single motor with some psychological measures.

The following are the hypotheses of the study:

1. Experimental masseter muscle pain does not alter the ability of individuals to perform isometric jaw-closing tasks.
2. Experimental masseter muscle pain leads to changes in recruitment patterns, thresholds of firing, or firing rates of single motor units, and changes in root mean square EMG activity, within the masseter muscle during standardized isometric jaw-closing tasks
3. Experimental masseter muscle pain leads changes in recruitment patterns, thresholds of firing, or firing rates of single motor units, or changes in root mean square EMG activity at one site within the masseter muscle during standardized isometric jaw-closing tasks that are different to any changes occurring at another site within the muscle.
4. Experimental masseter muscle pain leads to changes in EMG activity at one or more sites within the masseter that are not consistent with the *Vicious Cycle Theory* and the *Pain Adaptation Model* theories of pain-motor interaction.
5. Changes in masseter muscle activity will be associated with different levels of psychological measures.

1. MAIN FINDINGS OF THESIS IN RELATION TO THE ABOVE HYPOTHESES

The principal findings of the present study are as follows. There was no significant effect of the experimental pain on the generation or fine control of isometric jaw-closing tasks which means that experimental masseter muscle pain does not alters the ability of individuals to perform isometric jaw-closing tasks. These findings support the first hypothesis. Furthermore, the data are not consistent with the proposals of the Pain Adaptation Model which proposes a decrease in closing force during experimental pain.

In terms of the 2nd hypothesis, experimental masseter muscle pain led to changes in recruitment patterns, but not firing rates or thresholds of single motor units, and not changes in root mean square EMG activity, within the masseter muscle during standardized isometric jaw-closing tasks. Therefore, this hypothesis was partly supported.

In terms of the 3rd hypothesis, there was evidence for differences in the change in recruitment patterns of single motor units at one site within the masseter muscle during standardized isometric jaw-closing tasks in comparison with the change in recruitment patterns of single motor units occurring at another site within the muscle. However, there were no significant differences noted in terms of thresholds, firing rates or RMS EMG activity levels. Therefore, there was some data supporting this 3rd hypothesis.

In support of the 4th hypothesis, in general, the findings were not consistent with the earlier models of pain-motor interactions, namely, the proposals of the *Pain Adaptation Model* and the proposals of the *Vicious Cycle Theory.* Rather the data provide support for more complex models of *Motor Adaptation To Pain* where the modified patterns of motor

unit activity as a response to pain could be an attempt to maintain motor output to achieve the defined task and to protect the jaw motor system from further pain or injury (Hodges and Tucker, 2009; Murray and Peck, 2007; Murray and Lavigne, 2014).

Although in general, no significant differences in thresholds of firing or firing rates of single motor units, and in root mean square EMG activity was found at the two different sites of the masseter muscle during the standardized isometric jaw-closing tasks, a considerable inter-individual variability in the motor response to pain was observed in the present study during the standardized isometric jaw-closing tasks (Chapter III, section 4) as well as in the EMG activity at the two different sites of the masseter (Chapter III, section 5). Such a highly individualized motor response to pain is also consistent with more recent models of pain-motor interactions which propose that motor behavior to pain is mediated at multiple levels of the nervous system and is substantially variable between different individuals (Murray and Peck, 2007; Hodges and Tucker , 2011).

Qualitatively, there were no significant associations identified between psychological variables and jaw motor activity in the present study and this is not supportive of the 5th hypothesis. This lack of a significant effect is likely attributable to the low psychological distress demonstrated by our physically healthy and psychologically well-functioning study samples and is in accordance with the findings of literature that experimental pain models are less likely to capture emotions and cognitions present in chronic pain patients (Lobbezoo, et al., 2006).

The novel findings of this study suggest that the nervous system employs a different motor unit recruitment strategy throughout the masseter muscle in order to achieve the same biting task dynamics in pain as in the absence of pain and this change in motor unit recruitment may be due to uneven distribution of synaptic input across the motoneuron pool during pain. The overall picture elicited from our findings is inconsistent with earlier theories of pain-motor interaction, namely, the *Vicious Cycle Theory* and the *Pain Adaptation Model* which omit the role of higher brain centers in sensorimotor interaction. Rather the data are more in line with recent models of pain namely the *Integrated Pain Adaptation Model* and the *Theory of Motor Adaptation to Pain* proposing individualized and complex motor responses to pain.

2. ASYMPTOMATIC PARTICIPANT GROUP

The data collected from the participants in terms of PCS, and DASS, have all provided low scores. The DASS scores (Mean range 0.88-0.94) were all markedly lower than the scores reported from a large sample of chronic pain patients (13.3: Range 9-14) (Nicholas et al., 2008). Further, the PCS scores ranged from 0 – 33 (mean: 9.5), and these scores are entirely consistent with previous reports of PCS scores in asymptomatic participants (Akhter et al., 2014). These data support the contention that all participants were not clinically depressed, anxious or distressed and had levels of pain catastrophizing consistent with the general population.

3. EXPERIMENTAL MASSETER MUSCLE PAIN

Hypertonic saline infusion into skeletal muscles and ligaments has been used for almost 70 years to study the sensory phenomenon related to muscle pain (Kellgren, 1938; Hockaday and Whitty, 1967). It is a reliable and safe method of provoking experimental muscle pain with no reported side effects after numerous intramuscular injections (Stohler & Lund, 1994; Svensson et al., 2001). It has been observed that injection of hypertonic saline into a jaw muscle results in an increase of pain which reaches its maximum intensity, and then decreases shortly after the infusion is stopped and declines to no pain within 5-10 minutes (Svensson & Arendt-Nielsen, 1995). Numerous studies have been performed till now, but none has reported any undesirable side effects of long lasting discomfort, hematomas or tissue irritation after intramuscular injection of hypertonic saline (Stohler et al., 1992).

In the present study, 5% hypertonic saline solution was successfully infused with the aid of an infusion pump into the deep central region of right masseter muscle of 17 participants. The mean pain intensity induced by infusion of hypertonic saline was associated with moderate pain intensity (3-6 cm on 10 cm VAS). None of the participants reported any undesirable side effects during and after the infusion. In one of the participants, a small lump developed around the site of infusion and this disappeared within an hour after the termination of infusion.

3.1 Perceived Pain Intensity

A continuous infusion of 5% hypertonic saline into the right masseter muscle at different infusion rates (2-9 ml/h), preceded by a bolus infusion of 0.2 ml over 20 s, induced

moderate pain intensity of 3-6 cm for a period of approximately 20 minutes. The level of pain was maintained relatively constant and there was no significant effect for the jaw tasks performed on the mean pain intensity during hypertonic saline infusion (see Table III-4). The technique used for the infusion of hypertonic saline was similar to that used in previous studies (Baad-Hansen et al., 2009; Sae-Lee et al., 2007; Sae-Lee et al., 2008a; Svensson et al., 1997; Wang et al., 2004, 2005; Akhter et al., 2014).

The perceived pain intensity was achieved by infusing different amounts of saline into the tested muscles of different participants. This variation among individuals might be due to the influence of local factors at the site of the infusion, as well as possible genetic and environmental factors on the complex process of pain perception (Mogil et al., 1999; Diatchenko et al., 2005). Differences in pain perception might also be influenced by psychological factors (Ochsner et al., 2007) or by the perceived controllability of the pain (Salomons et al., 2007). The perceived controllability appears to be different between individuals and the neural mechanisms involved may be driven by executive processes in the prefrontal cortex, rather than lower regions associated with the properties of the stimulus (Salomons et al., 2007). Anxiety and fear are the most common psychological factors which affect pain perception and it has been found that fear of pain and anxiety influence pain perception either by modulating responses in pain processing systems, or by modulating intentional and emotional processes (Ochsner et al., 2007).

Moreover, pain experiences are multifactorial and variations in anatomical form e.g. variations in internal muscle architecture, muscle fibre types and variations in the neural

organization of the sensorimotor system, are likely to be additional factors that might give rise to the variation of infused volume between individuals. It might be possible that the infused volume into the muscle might have directly activated some sensitized nociceptive afferents (Sae-Lee, et al., 2008b; Shimada, et al., 2013). Nociceptive afferents may become sensitized by the needle insertion and may have caused the concomitant excitation of low-threshold mechanosensitive receptors which converge with nociceptive afferents onto WDR neurons in the brainstem (Türp, et al., 2002). A lower volume of solution therefore may have been needed to evoke moderate pain. Additionally, it is likely that sensitization of nociceptors provoked pain by muscle contractions during performance of jaw tasks and therefore needing less volume of solution injected (Türp et al., 2002).

The mean VAS scores reported by the participants during the performance of the ramp task (4.0±0.4) and the step task (4.1±0.8) were consistent with the pain ratings (Svensson et al., 1998b; Sae-lee et al., 2008; Sae-Lee et al., 2007) obtained from previous studies. Our results are also comparable with the findings of Stohler and Lund (1995) in which mean pain intensity of 4 cm on a 10 cm VAS was achieved by using a feedback-controlled infusion system.

Further, the analysis showed a statistically significant difference ($p = 0.00$) in the rating of pain intensity during the hypertonic saline (4.1±0.8) in comparison with the isotonic saline infusion (0.4±0.5) during the step tasks and similarly, for the hypertonic saline (4.0± 0.9) in comparison with isotonic saline (0.7±0.9) during the ramp tasks. These

results are entirely comparable to the findings of previous studies from our research unit (Sae-Lee, et al., 2008b; Sae-Lee et al., 2007; Minami et al., 2013; Akhter et al., 2014; Nash et al., 2010) and others (Svensson et al., 1997; Svensson et al., 1998; Wang et al., 2004) where injections of isotonic saline into the masseter muscle produced little or no pain compared to hypertonic saline. Hypertonic saline is a potent nociceptive stimulus and activation of nociceptors also leads to a release of algesic substances (*e.g.*, lactate, glutamate, prostaglandin E2) which can produce muscle pain in human (Mense 1993). Hence we argue that the increased intensity of self-reported pain was pain-specific as it was related to the persistence of pain caused by tonic infusion of hypertonic saline and not to infusion of the same amount of isotonic saline.

Infusion of isotonic saline (0.9% NaCl) has been shown to cause little or no pain in several studies (Stohler and Lund, 1994; Graven-Nielsen et al., 1997a, b; Svensson et al., 1998b). However, in some participants, infusion of isotonic saline was not totally pain-free and one participant rated the pain 2.6 cm on 10 cm VAS with the infusion of isotonic (see Table III-6) and this finding in our study is consistent with previous studies of injections of algesic chemicals into masseter muscles (Svensson et al., 2005; Shimada et al., 2013). There are many reasons which can induce this high level of pain in one participant e.g. mechanical irritation by needle insertion causing activation of nociceptive afferents or by increase intramuscular pressure within a confined anatomical compartment of masseter during performance of jaw tasks (Graven-Nielsen et al., 1997a;

Hannam and McMillan, 1994). The findings of the current study show that isotonic saline infusion situation is not a perfect control given that mild pain can be evoked.

3.2 Distribution of Pain

In current study, discrete pain areas were recorded after simultaneous injection of hypertonic saline into the masseter muscle, to assess the spread of pain and a visual inspection revealed pain was localized and confined to the injection site in most of the participants (15/17). However, 2 participants showed pain not only localized to the injection site but also there was referral of pain to the angle of the mandible and to the temple region which is consistent with previous studies (e.g., Stohler et al., 1992; Stohler and Lund, 1995; Svensson et al., 1996b; Graven Neilson et al., 1997, Thomas et al., 1997; Minami et al., 2013) where the infusion of hypertonic saline into the masseter muscle evoked pain that was localized arising from the injection site, and also the pain was referred to the adjacent areas on the ipsilateral side of the temporal region, in or around the TMJ, around the ear, the temporal region or sometime referred to the posterior teeth or mandible (Stohler et al., 1992; Stohler and Lund, 1995; Svensson et al., 1996b). Our finding of localized pain (originated from the injection site) during infusion of hypertonic saline in the right masseter muscle (see table III-7) was also similar to the findings of Hodges and Tucker in limb study (Hodges et al., 2009).

Experimental muscle pain induced by intramuscular injection of hypertonic saline, either as a bolus or as a continuous infusion was originally described by Lewis and Kellgren

(Kellgren, 1938, 1939; Lewis, 1938) who designated a standard model for inducing muscle pain by depolarizing group III and IV afferents in human participants (Graven-Nielsen et al., 1979; Svensson et al., 1998, 2000). According to these earlier studies, the muscle pain is generally described as a localized pain but could also be referred, and can sometime perhaps be confused with pain arising from other deep structures such as the underlying joint or ligaments.

The differential local and evoked pain from muscle and skin are probably caused by peripheral as well as central mechanisms whereas referral of pain after noxious muscle stimulation is suggested to depend primarily on central mechanisms. Referral of pain from one tissue to another is a common clinical phenomenon in many musculoskeletal and visceral pain disorders and the underlying mechanism is thought to be due to convergence of peripheral afferents from skin, muscle, viscera onto common central neurons (Feinstein et al., 1954; Foreman et al., 1979). Extensive convergent input from TMJ, muscle, pulp afferents via craniofacial nerves to cutaneous nociceptive neurons onto second order neurons in subnucleus caudalis could help explain the poor localization and referral and spread of pain involving the deep musculature (Sessle B.J 2006). Furthermore, unmasking of new receptive fields due to central sensitization could mediate referred pain (Mense et al., 2001).

Another possible mechanism for pain referral may be that a large volume of infused solution may cause activation of the number of nociceptors thereby increasing pain by spatial summation (Price et al., 1989; Nielsen and Arendt-Nielsen, 1997). There is

extensive convergence onto wide dynamic range (WDR) and nociceptive specific (NS) neurons within the brainstem (Lund and Sessle, 1994; Racich 2005; Sessle B.J 2006). Therefore, the central divergence of nociceptive input from masseter muscle nociceptive afferents as well as nociceptive or non-nociceptive afferents from other orofacial tissues onto WDR neurones may be a reason why pain could be referred from one site to other remote regions (Sessle B.J 2006; Sae-Lee et al., 2008; Svensson et al., 2005). Initially it was observed that the phenomenon of referred pain was related to the pain intensity (Stohler et al., 1992; Graven-Nielsen et al., 1996 Jensen & Norup, 1992) but recent literature have failed to confirm any significant correlation between perceived pain intensity and pain referral areas in the trigeminal system (Svensson, et al., 2003a; Schmidt-Hansen, et al., 2006). The reason for the lack of correlation between pain intensity and distribution of pain in the present trigeminal study is not known but could be due to a relatively small variation in VAS pain scores or differences between spinal and trigeminal processing of nociceptive information.

On the other hand, the pain areas drawn by participants who reported pain with isotonic saline infusion were very small (S10, S11) and did not cross the boundaries of the infused masseter muscles. The reason for mild pain evoked by isotonic infusion in some of the participants might be the activation of mechanosensitive nociceptors by the infused volume of saline (Türp, et al., 2002; Sae-Lee, et al., 2008a). None of the participants reported referral of pain to a remote site during isotonic saline infusion which is consistent with previous studies (Minami et al., 2013; Akhter et al., 2014).

3.3 Description of Experimental Masseter Muscle Pain from McGill Pain Questionnaire

We evaluated the sensory, affective, evaluative, and miscellaneous effects of experimental pain on the total pain rating index (PRI) by using the McGill Pain Questionnaire (Melzack, 1975). Experimental masseter muscle pain induced by hypertonic saline significantly affected miscellaneous, sensory and evaluative dimensions of the MPQ as compared to the isotonic saline control (see Tables III-8, III-9, III-10). This finding of the current study and the family of words used to describe the hypertonic saline-evoked pain sensation in the jaw muscles was in accordance with previous descriptions of both experimental and clinical jaw-muscle pain, (Stohler et al., 1994; Svensson et al., 2001; Türp et al., 1998) and with the findings of a previous masseter study (Sae-Lee, et al., 2008b).

The sensory word descriptors *i.e.*, "*sharp (53%)*", "*pinching (29%)* "and "*hurting (29%)*" were the most frequently chosen words in our tonic experimental pain study and this finding is also similar to the study by Sae-Lee et al. (Sae-Lee et al., 2008a) who have reported "*sharp*", and "*aching*" as the most selected sensory descriptors during tonic infusion of hypertonic saline. The Pain Rating Indices for the affective (scale score=2.2, weighted score=4.2) and evaluative (scale score=6.0, weighted score=6.1) dimensions were smaller compared with the miscellaneous (scale score=7.3, weighted score=8.2) dimension and this finding is in accordance with the study of Svensson (Svensson et al., 2003). It is also noteworthy that the increased frequency of using the term "*penetrating*", "*squeezing*" *and* "*spreading*" during the miscellaneous description of both hypertonic

and isotonic saline pain in relation to other dimensions may be related to the spreading of the fluid in the muscle and the most (see table III-8). Moreover, the prevalence of the word "*spreading*" (6 to 23%) did not match the occurrence of referred pain in masseter as reported by Svensson (40% to 87%) (Svensson et al., 2003)well and this gives confirmation of our findings about distribution of pain where most of the participants drew pain maps that reflected localized spreading around the site of injection (i.e. in the region of the masseter muscle).

Previous data have shown similarities in terms of the intensity, the sensory and the affective experience of experimental pain with the clinical chronic pain condition (Stohler & Kowalski, 1999). We found that the sensory pain rating index of the MPQ was higher (Table III-10)than the other categories (affective and evaluative) in both hypertonic as well as isotonic infusion and this supports the notion that the experimental pain is an appropriate model of the sensory-discriminative characteristics of persistent clinical pain conditions (Castrillon, et al., 2008). The increased frequency of using the term "*sharp"* by approximately 53% of participants to describe hypertonic and 23% of participants to describe isotonic was also in accordance with previous descriptors employing the same paradigm for injections into the masseter muscle (Sae- Lee, et al., 2008b Svensson *et al.* 1998b). Several features of experimental jaw muscle pain evoked by injection of hypertonic saline are similar to the clinical features of persistent TMD pain where spread and referral of pain to the temporomandibular joint, mandible, and teeth are reported (Stohler CS, et al 1999; Svensson P, et al 2001). Hence, human experimental pain models

can be used for studying the spread and referral of pain following noxious stimulation of different jaw muscle sites.

3.3.1 Hypertonic Saline Infusion vs. Isotonic Saline Infusion as an Important Comparison

The advantage of using isotonic saline infusion as a control is that it allows us to control for the volume of the solution injected with hypertonic saline and generally isotonic saline infusion does not induce moderate or severe muscle pain intensity. We found that participants described their sensory experience as "*sharp" ,"pricking", "pinching* "and "*sore"* associated with isotonic saline injection which is in agreement with previous descriptors that isotonic saline infusion may lead to a subjective experience described as pressure or tautness (Türp et al., 2002; Sae-Lee et al 2008). The order of infusions (hypertonic and isotonic) was alternated from participant to participant in order to reduce the possibility of order or temporal effects on the EMG activity. Initially, the recording of baseline trials (without infusion) allowed the participants to become accustomed to the tasks. The analysis in the present thesis will mainly focus on an analysis of motor effects during hypertonic saline vs. isotonic saline infusion, although comparisons will also be made to the baseline condition. There were significant differences in pain intensity score and pain rating indices for comparisons of hypertonic and isotonic infusions (see table III-8, table III-9). Therefore the analysis of motor effects during hypertonic saline infusion in comparison with isotonic saline infusion represents studying the net effect of pain on motor activity, given that identical procedures were carried out under both infusion conditions except for the presence of pain. Therefore the findings of the present

study can be compared with previous studies, for example in the jaw motor system which only collected data during hypertonic and isotonic saline infusion (Minami et al., 2013) or in the spinal motor system (Tucker et al., 2009, Hodges and Tucker, 2011) which only compared baseline vs. hypertonic saline.

3.3.2 Limitations of the Infusion Model

Many features of the current study that are reported above (e.g. pain intensity scores of about 4/10, pain areas over masseter, pain referral features, McGill descriptors) can all be features of patients' symptoms with TMD. Therefore our data provide support for the experimental pain model that is relevant to understanding what might happen in patients with TMD and to provide more insight into the spatial and sensory aspects of pain which encompass pain intensity, location, spread and referral of pain. Experimental pain models have been used for animal studies (Ness & Gebhart 1990; Le Bars et al., 2001) and human studies and are quite advantageous in various preclinical investigations of pain. Moreover, with these models, the investigators can control pain features (intensity, duration, location etc.) and provide quantitative measure of psychophysical and behavioral responses (Graven-Neilson et al., 2001; Drewes et al., 2003). However, pain has a complex multidimensional nature and experimental models record neuronal nociceptive activity, electrophysiological activity, or behavior (Sengupta & Gebhart, 1994) which do not reveal all aspects of pain processing that involve many different parts of the central nervous system (Le Bars et al., 2001). Thus although experimental pain models have served an important role in understanding and translating information to clinical pain studies, a number of differences still exist comparing healthy participants

with TMD patients, for example, in terms of psychological measures such as catastrophizing.

The experimental pain models are usually carried out in healthy young participants who have, usually, low scores on psychological distress measures such as depression, anxiety and stress. Measures of distress are usually higher in patients with chronic pain conditions. Further, *"The chemical stimulation methods all have a problematic reproducibility with large inter-individual differences"* (Mørk et al., 2003). For example, these chemical stimulants may require higher doses to activate nociceptors in non-inflamed tissue compared to the situation in inflamed tissue.

In human experimental pain studies, it has been found that intramuscular injection of hypertonic saline cause delayed onset muscle soreness (DOMS) and referred pain more often and over a wider pain area than injection to normal muscle (Graven Neilson et al., 2006). Delayed onset muscle soreness is primarily considered to be due to changes in muscle tissue and sensitivity of peripheral nociceptor (K. Mizumura et al., 2009). On the other hand, imposing the eccentric contraction of jaw closing muscles as suggested by Turker et al can cause DOMS which can be used as an alternative to use of hypertonic saline injection and could be clinically more relevant to TMD (K Turker et al., 2010).

4. SIMULTANEOUS RECORDING OF JAW MUSCLE EMG ACTIVITY UNDER STANDARDIZED ISOMETRIC JAW TASKS DURING EXPERIMENTAL MASSETER MUSCLE PAIN

We refined a technique for the simultaneous recording of EMG activity from 2 sites within the masseter muscle during standardized isometric jaw tasks together with the well-established experimental muscle pain model. This model has allowed us to study the effects of experimental jaw muscle pain on isometric jaw tasks. The within-participant design of this methodology provides a powerful tool to assess EMG activity and jaw force generation from the same participant in different experimental conditions i.e., baseline control, hypertonic saline infusion and isotonic saline infusion. All participants were able to perform the 3 sessions of the experimental trials.

4.1 Effect of Experimental Masseter Muscle Pain on Isometric Force Jaw Tasks

The present methodology of recording ramp and step isometric tasks under experimental pain allows an extension of studies of the effects of pain on jaw motor activity. Previous studies from our and other laboratories have focused on the effects of experimental pain on jaw movements (Sae-Lee et al., 2008; Akhter et al., 2014; Svensson et al., 1996; Svensson, et al., 1997; Matre et al., 1998; Wang et al 2005; Wang et al 2000; Stohler et al., 1999). There have been fewer studies of the effects of experimental masseter muscle pain on isometric jaw tasks (Minami et al., 2013; Sohn et al., 2004; Sophie et al., 2003; Turkawski, et al., 2001; Shimada et al., 2013; Arima et al., 2013; Terebesi et al., 2015) and therefore, the modulation of human SMU firing rate and recruitment pattern by experimental muscle pain during isometric contraction has not been described in detail. Hence, the present methodology allows a rigorous testing of the general applicability of earlier theories explaining the effects of pain on motor activity, namely, the Pain Adaptation Model and the Vicious Cycle Theory, by extending the range of jaw motor tasks (namely, ramp and step tasks) and jaw muscle sites (2 sites within the masseter

muscle) than have been employed in the past. As in previous studies (Sae-Lee et al., 2008; Akhter et al., 2014; Memon, M. S. et al., 2013; Wang et al., 2000; Sohn et al., 2000), the participant is motivated to achieve the same jaw motor parameters during experimental jaw muscle pain as during the pain-free or isotonic control conditions. It is considered that this approach may provide valuable new insights into how the jaw motor system operates in chronic muscle pain where the individual must achieve specific motor targets in order to function, for example, clearly articulated speech, sufficient chewing ability, etc.

In the present study, there was no significant effect of the experimental pain on the ramp or the step task performance. In all participants, there was no significant difference for the step level 1 and step level 2 force levels under the hypertonic saline infusion in comparison with the isotonic saline infusion. Therefore, despite the presence of pain, all individuals were able to perform this 2 step task in experimental masseter muscle pain and were able to maintain the same force levels. These data are not consistent with the Pain Adaptation Model which would propose a decrease in closing force during experimental pain. Further, there was no effect on the ability to perform the 2 step levels task which requires a level of fine control in order to generate the 2nd step level which was slightly greater than the first step level. These findings indicate that not only did pain have no effect on the ability to generate the jaw closing forces in these tasks but also there was no effect on the fine control of jaw closing forces.

While consistent with our recent study (Minami et al., 2013; Michelotti et al., 2014; Abhishek et al., 2013) showing no significant effect of experimental masseter muscle pain on the ability to generate force to a single target level, the present findings contrast with our previous findings of significant effects of experimental masseter muscle pain on jaw opening amplitude and/or velocity during standardized open-close jaw tasks (Sae-Lee et al., 2008) and during repetitive open-close jaw movements (Akhter et al., 2014). It should be pointed out however, that the jaw movement tasks reported in these earlier studies are quite different from the isometric closing tasks carried out in the present study. The open-close jaw tasks (Sae-Lee et al., 2008) showed reductions in amplitude of opening (closing not reported in that study). Opening in this task is generated by the jaw opening muscles, lateral pterygoid and digastric, and not the masseter. In the other study involving a repetitive open-close task, significant effects of experimental masseter muscle pain were noted on opening *and* closing amplitude and velocity (Akhter et al., 2014). As previously noted (Murray and Peck, 2007), it is possible therefore that the nature of the task plays an important role in determining whether pain effects are noted.

Furthermore, the present findings are not consistent with the many studies both in the spinal and trigeminal literature showing significant reductions in movements and/or agonist muscle EMG activity during experimental or clinical pain in comparison with controls (Svensson et al., 2001; Lund et al., 1991; van Dieën et al., 2003; Stohler et al., 1999; Lund et al., 1994; Lund et al., 2001; Graven-Nielsen, 2003; for review Murray and Peck, 2007; Shimada et al., 2013). Clear evidence for inhibition of agonist muscle EMG

activity, in comparison with pain-free controls, has been particularly noted during the generation of maximum voluntary contraction forces.

The present data show that the masseter muscle pain had no significant effect on the ability to generate isometric closing forces at 2 step levels and under ramp conditions and these findings are consistent with our previous study (Minami et al., 2013). One possible reason for the absence of a pain effect in the present study could relate to the lower forces employed in the present task and this is consistent with SMU study in hand muscle where Birch et al (2000) found no significant effect of experimental muscle pain on firing characteristics of SMU at low force levels (Birch et al., 2000). It is possible that the effects of pain on motor activity as proposed by the Pain Adaptation Model may only become apparent at high force generation. However, the open-close tasks affected by pain in the previous studies from the present laboratory (Sae-Lee et al 2008; Akhter et al., 2014), were not performed at maximal force generation.

Therefore, the data do suggest that while some jaw motor tasks are affected by experimental jaw muscle pain in a manner consistent with the proposals of the Pain Adaptation Model (Sae-Lee et al., 2008; Akhter et al., 2014; Svensson et al., 2000; Madeleine et al., 1999a, b; Turp et al., 2002; Sohn et al., 2000; Shimada et al., 2013), other tasks do not appear to be so affected (at least in terms of the ability to generate 2 force levels at low forces). Taken together, these findings lend support to more complex models of pain-motor interactions where the nature of the task is a factor in determining

whether a motor effect is observed with pain (Hodges and Tucker, 2009; Murray and Peck, 2007; Murray and Lavigne, 2014).

4.1.1 Variability of Force among Individuals

The level of biting force was different among different participants (Table III-11). In some participants (9, 13) force levels were very low whereas in some participants it was very high (3, 17). Many factors can be considered for this including the difference in anatomy of muscle, pattern of biting, state of occlusion and pain catastrophizing behaviour of the participants. For example, it has been observed that cross-sectional thickness of the masseter differs between individuals with different craniofacial morphology and it has been expected that participants differ in pain, fatigue, and endurance during the performance of prolonged static and dynamic tasks due to diversity in neuromuscular features along with different vertical craniofacial morphology (Farella et al., 2003). Another reason of difference in biting force among individuals is that the target force level was selected based on the firing rate of SMU's.

4.2 Effect of Experimental Pain on Recording of Single Motor Unit Activity at Two Sites within the Masseter Muscle during Standardized Isometric Jaw Force Task.

The changes in muscle activity during nociceptive afferent inputs have been thought to be governed by the predictions of the Vicious Cycle Theory (Travell et al., 1942) or the Pain Adaptation Model (Lund, et al., 1991) which propose either a uniform increase or a uniform decrease in the behavior of the whole muscle. The former theory predicts an increase in the activity of a painful muscle, irrespective of its function in the task while the latter model is based on the premise that pain inhibits the activity of muscles that

produce painful movement (agonist) and facilitate opposing muscle activity (antagonist). Although some observations in the clinical and experimental literature appear to be consistent with their predictions (Lund et al., 1991; Graven-Nielsen et al., 1997; Svensson et al., 1996, 1997, 1998; Turp et al., 2002), these theories do not seem to explain the wide range of changes in muscle activity that can occur in pain. In this section, our findings will be interpreted in the view of the predictions of previous theories and with reference to more recent models that have been proposed of the pain motor interaction.

The single motor unit activity at two different sites within the masseter muscle was investigated during the performance of the standardized biting task under different experimental recording sessions. The results were compared between no pain (baseline) and pain (hypertonic saline infusion), and between pain (hypertonic saline) and control (isotonic saline) conditions to elicit the net effect of pain on muscle activity at two different sites.

4.2.1 Effect of Experimental Masseter Muscle Noxious Stimulation on the Occurrence of SMU Activity

Tables III-12 and III-13 summarize for each masseter site the occurrences of SMUs during the ramp and step tasks under baseline, isotonic and hypertonic infusion conditions. In most of the SMUs recorded (14/26 (54%) at RMI/RMP site; 18/24 (75%) at RMS/RMA site; 32/50 (64%) at both sites), the presence of hypertonic saline in comparison with isotonic saline resulted in no effect on the occurrence of a SMU. These proportions of SMUs present under both HS and IS conditions are approximately

comparable to the proportions from our previous study of isometric jaw tasks in the masseter, where 21/36 SMUs (58%) were present during 1 or more trials of both HS and IS infusions (Minami et al. 2013). However, both sets of studies differ from the findings in another study of the quadriceps and flexor pollicis longus muscles, where 38% and 26% of SMUs, respectively, were recruited under both baseline and HS infusion conditions (Tucker et al., 2009; there were no IS controls in this study); the remaining units were either recruited or de-recruited during pain in comparison with baseline. Taken together, the data suggest that there may be differences in the proportions of SMUs that remain recruited under pain and no-pain conditions between jaw muscles and limb muscles. Other findings have also demonstrated various differences in the properties of single motor units between masseter and limb and trunk muscles, including the size of motor units, type of muscle fibers and the presence of myosin heavy chain isoform (MHC) (Palgar et al., 1973; Morris et al., 2001; Soussi-Yani 1990; Stal P. et al., 1994).

Therefore, pain did not affect the occurrence of most SMUs during the ramp task and the step task at both sites. *The Pain Adaptation Model* proposes that during pain, agonist muscle activity is reduced in the generation of forces and this inhibition operates at the brainstem level through local inhibitory reflex circuits. The present data show that any inhibitory effects on α-motoneuronal activity from nociceptive activity (as proposed by *the Pain Adaptation Model*) were insufficient to prevent the descending drive, from the face area of the primary motor cortex, from activating and recruiting many of the SMUs required during these two tasks. The goal-directed nature of both tasks therefore was able to override any possible local brainstem inhibitory effects as might arise from *the Pain Adaptation Model*. The absence of an effect on the occurrence of many SMUs is not

consistent with the proposals of *the Pain Adaptation Model*. Nonetheless, there were SMUs that were present during isotonic saline infusion that were not present during hypertonic saline infusion. This finding is indeed consistent with *the Pain Adaptation Model* (see below) and, furthermore, is consistent with other recently reported studies where noxious masseter or tongue muscle stimulation results in inhibitory influences at the level of primary motor cortex (Adachi et al., 2008; Nash et al., 2010).

A possible explanation for the finding inconsistent with pain adaptation model (i.e. pain did not affect the occurrence of most SMUs), might be that as bite force magnitude increases, the number of different neural strategies possible for the task to be performed decreases, and this might result in any inhibitory effects on the occurrences of SMUs, as proposed by the Pain Adaptation Model, might become less likely to be manifest. However, low forces were needed in the present tasks. These observations were noted at both sites within the masseter muscle and suggest that the ability to override any inhibitory effects on SMU activity during tasks is a generalized feature throughout the masseter muscle. Clearly, more studies are needed to confirm this tentative conclusion.

In comparison with isotonic saline infusion, there could be a recruitment of new SMUs during hypertonic saline infusion, or a de-recruitment of SMU activity. The de-recruitment of SMU activity was apparent at both sites (6/26 de-recruitments at RMI/RMP site; 2/24 de-recruitments at RMS/RMA site), that is, SMU activity was present during task performance under isotonic infusion, but was not present during hypertonic saline infusion. These data are entirely consistent with the proposals of *the*

Pain Adaptation Model that nociceptive activity would act via local circuits to inhibit α-motoneuronal activity (Lund, et al., 1991; Murray and Peck, 2007). The ability to continue to perform the ramp and step tasks during the hypertonic saline infusion despite the cessation in activity from a SMU means that other SMUs need to be recruited either at the same sites or at different sites within the masseter or other jaw closing muscles to allow successful performance of the task.

Other mechanisms also exist for maintaining force despite the cessation in firing of a SMU. For example, potentiation of twitch force in masseter motor units has been shown to be a pain-related compensatory mechanism to maintain constant force output during painful isometric contractions when SMU firing rates decrease (Sohn et al., 2004; Turkawski and vanEijden, 2001). Thus, there may have been potentiation of the twitch force of SMUs that were active during both hypertonic saline and isotonic saline infusion with the purpose of maintaining constant force output.

In comparison with isotonic saline infusion, there could be a recruitment of new SMUs during hypertonic saline infusion. At both sites, there were 6 (12% of total of 50 SMUs) newly recruited single motor units during HS infusion that were not present during IS infusion. This data supports the findings of pain studies in limb muscle and in masseter motor units recordings that the pattern of recruitment of single motor units is not always the same (Tucker and Hodges, 2009; Minami et al., 2013).

In a previous study, 10/36 (28%) SMUs were newly recruited during HS in comparison with IS infusion (Minami et al. 2013). The reason for the fewer occurrences of newly

recruited SMUs during pain in the present study is unclear. One possibility might be the difference in the location of the electrodes. In the previous study, electrodes were placed at the deep central region of the masseter muscle whereas in the current study, the electrodes were not placed in the deep central region of the masseter but were placed at the superior/anterior (RMS/RMA) and inferior/posterior (RMI/RMP) (superficial) sites of the muscle. In most of the participants, therefore the electrode locations were closer to the origin and insertion of the masseter. As the masseter has a complex architecture and a heterogeneous function (Blanskma et al., 1992), it is possible that the deep central region of the masseter has different fibre directions as well as possibly different functional roles compared to the superior and inferior regions of the muscle (Schumacher, 1961; Baron and Debussy, 1979) and selective activation of fibre within these different regions may generate forces in different directions. In addition, the literature has shown differences in activity at different parts of a muscle during the same task (Romeny et al., 1982; Hoffer et al., 1987; Blanksma et al., 1992).

Furthermore, when the results are compared with previous study, another possible reason might be the location of the infusion needles. In the previous study (Minami et al., 2013) the electrodes were placed in the centre of the muscle which was proximal to the infusion needle and the neural input in that part of muscle would be enough to activate motor units of that region. On the other hand, in the current study, the infusion needle was placed in the centre of the muscle and the electrodes were distant from the infusion site and it might be possible that nociceptive input at the part of muscle with electrodes (far from the infusion needle) would not be enough to activate motor units.

The recruitment of new SMUs during hypertonic saline infusion may have occurred in order to allow the task to be completed because SMUs at the same site or other sites (not recorded) were inhibited during the hypertonic saline infusion. There was only 1 possible site where there was evidence in the present study of recruitment of a new SMU at the same site at which a SMU ceased to be active, and that is site 6 (Table III-10). At this site, SMU "Z" was recruited during hypertonic saline infusion but was not present during isotonic saline infusion, while SMU "b" was not present during hypertonic but was present during isotonic infusion. The data suggest that, in agreement with results of other studies, that there is a highly compartmentalized and task dependent specialization of motor unit recruitment in different subdivisions of muscles (Hoffer et al., 1987; Herring et al., 1989; Blanksma et al., 1992).

4.2.2 Analysis of Possible Carry over Effects

The presence of most of the newly recruited SMUs in the isotonic saline sessions (4/6) along with hypertonic/pain sessions might be a possible carry over effect of the pain which could cause a SMU to remain active in the next recording session of isotonic infusion if it became initially recruited in the hypertonic saline session. In all participants where a SMU was active during isotonic saline infusion as well as hypertonic saline infusion but not baseline, the hypertonic saline infusion was the first infusion in these participants. Another possibility is that the low level of pain generated by the isotonic saline during the standardized biting tasks might be sufficient to produce significant effects on motor activity observed in the present study, and thereby to maintain recruitment of a new SMU that was also present during hypertonic saline infusion. As this difference was noted in step 1 task and the amount of force required to perform step 1

task was very low, it might be possible that the unit which become active during hypertonic session maintain the tonic activity during isotonic infusion session as well.

A comparison of the different jaw tasks to see the change in the pattern of occurrence of single motor units during hypertonic and isotonic showed no significant difference in the pattern of occurrence of single motor units during ramp and step 2 task but a difference in pattern of occurrence of SMU at step 1 was observed (see Table III-16). The possible reason for this relate to the amount of force required to perform the different jaw tasks. Thus, the amount of force required to perform step 2 of the step level task was higher than the step 1 of the step level task. The pattern of occurrence of SMUs during the step 2 might therefore be more similar to the pattern observed during the ramp task which also involved a higher force level towards the end of the ramp task than at step 1. However, the comparison of the ramp and step 1 of the step level task, might be more likely to show a difference in the pattern of occurrence of SMUs at both sites of masseter because of the difference in force required to complete the task (i.e. less force required for step 1 task). Hence we conclude that in order to find the difference between hypertonic and isotonic infusions we need to look at higher force/max levels however it is difficult to record firing rates at that stage because of multiunit activity present at that higher force levels.

4.2.3 Comparison of Occurrence of SMUs at Two Different Sites of Masseter

There was a suggestion, for the comparison of hypertonic saline vs. isotonic saline infusion that at the inferior/posterior (RMI/RMP) masseter site, there was more likely to be recruitment or de-recruitment of SMUs than at the superior/anterior (RMS/RMA) site.

These data are summarized in Tables III-19, III-20 and III-21. Insofar as the comparison of hypertonic saline infusion with isotonic saline infusion represents a valid assessment of the net effect of pain (see section 3.2.1 for rationale), the data suggest that there may be differences between the superior/anterior (RMS/RMA) site in comparison with the inferior/posterior (RMI/RMP) site in the degree with which SMUs are recruited or de-recruited in pain. Thus the data indicated that there was significantly (chi-square; $p<0.05$) more likely to be recruitment or de-recruitment of SMUs during pain at the RMI/RMP masseter site (9:17), than at the RMS/RMA site (3:21). These data suggest therefore that while reorganization of SMU activity may be feature of the effects of pain on motor activity as proposed both in the trigeminal and spinal systems (Murray and Peck, 2007; Hodges and Tucker, 2009; Murray and Lavigne, 2014), the pattern of reorganization at least in terms of recruitment and de-recruitment of SMUs may vary throughout the masseter muscle (Schindler HJ et al., 2014). This finding has implications for understanding the effects of pain throughout other jaw muscles and may have implications for development of future studies of pain effects in other muscles, such as lower back and limb muscles.

The presence of such a varied effect of pain throughout the masseter muscle might be related in some way with the functional heterogeneity that has been demonstrated in this muscle (Hannam and McMillan, 1994; Blanksma et al., 1992; van-Ejiden 1998; Review Burke 1986). For example, the relative contribution to the tasks may vary with the site in the masseter muscle. This is consistent with a recent study of Schindler who found task dependent recruitment of MUs in different sub-volumes of masseter (Schindler HJ et al.,

2014). In comparison with SMUs located at the right masseter inferior/posterior (RMI/RMP) site, SMUs located at the right masseter anterior/superior (RMS/RMA) site were less likely to be recruited or de-recruited during hypertonic saline infusion in comparison with isotonic saline infusion. It is possible that motor units located at this superior/anterior (RMS/RMA) site may be more resistant to changes in recruitment or these motor units may be more essential in the performance of these particular closing tasks. Clearly, the limited sample size calls for more detailed studies to explore this potential differential effect.

4.2.4 Comparison between Sites In Terms Of Recruitment Patterns for Ramp vs. Step Tasks

Table III-20 and III-21 summarises whether the presence or not of a SMU under hypertonic saline infusion and isotonic saline infusion was the same under the ramp task in comparison with the Step 1 task, and the ramp task in comparison with the Step 2 task. There was a larger number of different patterns between the ramp vs. Step 1 (3:9) and ramp vs. Step 2 tasks (3:2) at the inferior/posterior (RMI/RMP) site in comparison with the superior/anterior (RMS/RMA) (0:3 and 0:0) site. These data suggest that the recruitment pattern of SMUs in pain is less influenced by the task within the superior/anterior (RMS/RMA) region than within the inferior/posterior RMI/RMP) region of the masseter.

There was suggestive evidence for a difference between the ramp and the Step 1 and Step 2 tasks in the presence or not of a SMU under hypertonic saline infusion and isotonic

saline infusion. Thus at both sites, there were SMUs that were active during both hypertonic saline infusion and isotonic saline infusion during the ramp task and during the Step 2 task but not during the Step 1 task. The occurrence of SMUs under both hypertonic saline infusion and isotonic saline infusion during both the ramp task and during the Step 2 task may reflect the higher forces demanded by these tasks in comparison with the Step 1 task where the individuals were instructed to hold the force at a level where one or more SMUs were just being recruited. At this Step 1 level, these SMUs will likely be under less cortical descending drive to maintain their activity and may therefore be more susceptible to any inhibitory influences from nociceptive activity. This finding of different MU activity among various jaw tasks (ramp vs. step 1 task0 is consistent with one of the recent findings of Terebesi who found differential MU recruitment behaviour in small sub-volumes of masseter in response to a small change in dynamic loading of mandible (Terebesi et al ., 2015).

4.2.5 Analysis of Pattern of Change in Activity In Relation To VCT and PAM

An analysis was done unit by unit and site by site to see if the data support the earlier models (vicious cycle theory and pain adaptation model). There was only little evidence for support for both of these earlier models. A qualitative comparison of two sites in the masseter muscle to see whether the pattern of occurrence of SMU activity was consistent with vicious cycle theory or pain adaptation model showed there were more effects not consistent with either VCT or PAM at the right masseter superior/anterior (RMS/RMA) site (Table III-23) in comparison with the right masseter inferior/posterior (RMI/RMP) site (Table III-24). These findings are consistent with comprehensive review of the chronic back pain literature, where the effects of pain on trunk muscle activation were

neither consistent with the vicious cycle theory nor with the pain adaptation model (van Dieen et al., 2003) and it has been mentioned that in determining the effect of pain on jaw motor activity, the tasks being performed is considered to be more important (Murray and Peck, 2007). Therefore, although the earlier models have many positive features, it appears that these theories cannot explain all effects of pain on jaw muscle activity.

The present study demonstrates that the presence of a significant difference in SMU activity between hypertonic and isotonic saline could vary with the tasks (ramp, step level 1 and step level 2) performed and in addition, the effect could vary from person to person. One example of this is that at RMI/RMP site (see Tables III-22) during step level 1 task where the pattern of occurrence of 4 SMUs (C, G, S, T) was consistent with the VCT, and the pattern of occurrence of 5 SMUs (H, I, J, O, U) was consistent with PAM. On the other hand, in the step level 2 task, the pattern of occurrence of 2 SMUs (S, T) was consistent with VCT and none was consistent with PAM (see Table III-22). The present study revealed data both consistent with and not consistent with each of these two models. In the case of the vicious cycle theory, the present findings of no significant effect of jaw muscle pain on the occurrence of most SMUs during different jaw tasks at both sites is not consistent with a critical component of the vicious cycle theory that pain leads to increased jaw muscle activity (Lund et al. 1991; Stohler et al., 1999; Svensson et al., 2001; van Dieen et al., 2003; Murray and Peck, 2007), or with The Pain Adaptation Model which proposes that agonist muscle activity is reduced during pain (Lund et al., 1991; Murray and Peck, 2007 Adachi et al., 2008; Nash PG et al., 2010).

These findings of little effect of pain on motor activity in terms of SMU occurrence during goal-directed tasks is similar to our understanding of how orofacial pain patients function in situations where motor performance cannot be compromised in certain situations e.g., speech and chewing. We propose that under goal-directed jaw tasks, higher brain centers can modify the patterns of change in SMU activity in order to maintain motor output to achieve the defined task. This might explain the increase in activity in some parts of muscle (Table III-12 and III-13) as proposed by the integrated pain adaptation model (IPAM) (Murray and Peck 2007) and also as proposed by limb and trunk studies (Hodges and Tucker, 2011) who suggest a re-organization of activity within the painful muscle, that is, a slowing and/or de-recruitment of one population of motor units and a recruitment of a new population of units (Tucker et al., 2009; Hodges and Tucker, 2011).

Analysis of our data also has implications for the development of future studies to investigate the effect of pain in jaw muscles and it argues for the need for re-assessment of management strategies for jaw muscle pain conditions based on earlier models of Vicious Cycle Theory and Pain Adaptation Model (Travell et al., 1942; Johansson and Sojka, 1991; Lund et al., 1991). Apart from proposing quite different effects of pain on motor activity, these earlier models propose all-inclusive increases or reductions in muscle activity during pain throughout a muscle. The data of the present study provides support for more recent models proposing a re-organization of the recruitment strategy

adopted by the brain in the control of motor units in the presence of pain (Murray and Peck, 2007; Hodges and Tucker, 2011).

5. ANALYSIS OF THRESHOLD VALUES OF SMUS AT BOTH SITES IN BOTH TASKS

The analysis of the mean threshold values of all sites for the ramp task and step tasks during all recording sessions was also performed for testing for any interactions between the infusion sessions and to see the difference in the threshold values of two different sites. There were no significant differences between sessions and no significant interactions for the threshold values at each of the two sites.

A previous study of SMU activity recorded under control and pain conditions (capsaicin infusion) during the performance of standardized isometric contractions has demonstrated a reduction in firing rates of SMUs without any change in recruitment threshold during pain (Sohn et al.,. 2000). Our present findings are therefore consistent with these previous findings and suggests that threshold of SMU firing is a robust feature of SMUs that remain active during pain in comparison with control. This means that the change in motoneurone discharge is not due to the direct effects of hypertonic saline on the motoneurons; this is consistent with animal literature (Iggo A. et al., 1961; Paintal AS et al., 1960) showing that hypertonic saline activates group III and IV afferents and does not have an effect on motor unit properties (Farina et al., 2004). one explanation for the finding that the recruitment threshold of the SMU did not seem to decrease during painful contraction in the present study, could be recruitment of additional SMUs (Table III-16

and III-17) and increase firing rate of some existing SMU's (Table III-35) in order to maintain constant force output without changing the recruitment threshold. However, as indicated below (SECTION 7), there was no evidence of a change in firing rate of SMUs during hypertonic saline infusion in comparison with isotonic infusion.

While this group assessment of SMU thresholds did not reveal any significant differences between HS and IS infusions, an individual analysis of SMU thresholds did indicate that, qualitatively, thresholds did not necessarily remain unchanged. Thus, for each SMU and each task, an analysis was carried out as to whether thresholds increased or decreased in each task for comparisons of HS vs. IS infusion. Thus, at the RMS/RMA site during the ramp task, 9 SMUs exhibited a decrease in threshold and 8 exhibited an increase in threshold during HS in comparison with IS infusion. During the step task, 7 SMUs exhibited an increase in threshold and 13 exhibited a decrease in threshold during HS in comparison with IS infusion. And at the RMI/RMP site during the ramp task, 5 SMUs exhibited a decrease in threshold and 8 exhibited an increase in threshold during HS in comparison with IS infusion. During the step task, 9 SMUs exhibited an increase in threshold and 14 exhibited a decrease in threshold during HS in comparison with IS infusion. These changes may relate to subtle variations in the rates of force change between individuals given that no individual can perform the task exactly the same way between trials. It is well-established that the thresholds of firing of SMUs in both the spinal and trigeminal systems are dependent on the rate of force change (Freund 1983).

Another possibility is that individual SMUs might be differentially affected in terms of threshold by the nociceptive stimulus. We have shown in previous studies of effects of HS infusion, in comparison with IS infusion, on standardized jaw tasks, that the effects of pain on overall muscle activity could differ between individuals, with some individuals showing increases in EMG activity during pain while other individuals showing a decrease in EMG activity (Sae-Lee et al., 2008; Shimada et al., 2013; Wang et al., 2000; Minami et al.,. 2013). These changes may reflect the complexity of re-organization of SMU activity within a muscle during pain and the effects may not be apparent with group analyses where all single motor units are grouped together – the increases and decreases simply cancel each other out. Further studies are needed to clarify this issue and this could involve repeating more trials of a task within a participant to determine whether the changes in individual SMU threshold are indeed significant.

5.1 Possible Changes in Recruitment Order in HS vs. IS

An analysis was carried out as to whether the sequence of recruitment of SMUs at a site within the masseter during the ramp and the step task was altered during the infusions (Tables III-26 and III-27). During both ramp and step tasks at both sites, most of the recruitment sequences of SMUs within each participant did not change between HS and IS. However, participant 7 for the ramp task and participants 4 and 6 for the step task exhibited a different recruitment sequence during HS in comparison with IS infusion at RMS/RMA site. Similarly, at RMP/RMI site participants 5 and 6 for the ramp task and participants 1, 2, 4 and 5 for the 2-step task showed a different recruitment order during HS in comparison with IS infusion.

These finding of individual differences (as shown above) in the presence of pain is consistent with previous studies that have suggested that pain interacts in a unique way in the individual with the complex and individualized organization of the sensorimotor system and pain experience (Raber P, et al., 2002; Mogil JS et al., 1999; Murray and Peck 2007 Sae-Lee, et al., 2008a; Sae Lee, et al., 2008b; Hodges, 2011; Hodges & Tucker, 2011; Wiesinger, et al., 2013). In addition, as we have found that individual differences of experience of pain were associated with multiple changes in motoneurone recruitment strategy within a region of muscle and hence, our data extend the previous findings how force can be maintained in presence of pain (Tucker, et al., 2009; Hodges et al., 2012; Minami, et al., 2013)

While this possible change in recruitment order in pain has not been previously reported in the trigeminal system in pain, similar findings have been reported in the limb literature (Tucker et al., 2009, 2010; Hodges et al., 2011, 2012; Madeleine, et al., 2006; Falla, et al., 2007b; Falla et al., 2008; Samani et al., 2009). Our findings may be better able to be illuminated by the new data emerging from the limb muscle studies which suggest that instead of uniform inhibition or excitation of muscle activity involved in the task, pain results in a reorganization of activity within muscles by changing the recruitment strategy of motor units (Tucker, et al., 2009; Tucker & Hodges, 2009). These other studies have observed changes in the activity of the motoneurone pool during pain including a cessation of discharge of one population of units, and the recruitment of another population of units, and provide evidence for an alternative mechanism for changes in motor control i.e. re-organization of SMU activity during force generation under pain in

comparison with control. Taken together, the data suggest that pain may have differential effects on different regions of the masseter.

6. ANALYSIS OF FIRING RATES

Overall there was no significant difference in firing rate of SMU when comparing the hypertonic vs. isotonic infusion session and when comparing the 2 sites (i.e. RMS/RMA vs. RMI/RMP) at masseter (See table III-35) which is consistent with the findings of Minami who found no significant difference in firing rates of MUs between pain (hypertonic) and no pain (Isotonic) sessions in masseter (Minami et al., 2013). However some SMUs showed increase (4 at RMI/RMP and 8 at RMS/RMA) and others showed decrease (5 at RMI/RMP and 3 at RMS/RMA) in firing rate during hypertonic infusion session in comparison to the isotonic infusion session which is in agreement with the current models for treatment of lower back pain (Hodges and Tucker, 2011). Moreover, individuals could show increase or decrease in firing rates in response to pain and at different sub-volumes of muscle.

The results of qualitative assessment of MU recruitment characteristic (firing rate) is in agreement with recent studies (Tucker and Hodges, 2011; Minami et al., 2013; Schilder et al., 2014; Terebesi et al., 2015; Sohn et al 2000) suggesting an altered motor unit activity in response to pain and to various jaw tasks and this finding may add to the understanding of mechanism behind the management of patients with masticatory muscle pain.

7. ANALYSIS OF ROOT MEAN SQUARE ACTIVITY AT BOTH SITES IN BOTH TASKS

Root mean square analysis of EMG activity from the masseter revealed no significant differences in the pattern of activity between HS and IS infusion at right masseter superior/anterior site (RMS/RMA) or at the right masseter inferior/posterior site (RMI/RMP). These findings are generally inconsistent with many previous studies showing that pain has does indeed have effects on overall EMG activity during a variety of tasks (Graven-Nielson 1997; Svensson et al., 1997, 2001, 2002; Wang et al., 2000; Farina et al., 2005; Stohler CS et al., 1996; Sae-Lee et al., 2008a, b; Tucker et al., 2009; Schulte E et al., 2004; Le Pera D, et al., 2001). We have previously provided evidence for a task dependency in the effects of pain on motor activity with some jaw movement tasks being unaffected by HS infusion, in comparison with IS infusion, while other tasks were affected (Sae-Lee et al., 2008a, b; Shimada et al., 2013; Majeda 2014; Maulina 2013; Memon 2013).

The data can also be interpreted in relation to the VCT and PAM which would propose that, in the presence of pain, an increase in activity in the case of the VCT or a decrease in agonist activity in the case of the PAM (Svensson P, et al., 2001; Stohler CS et al., 1999; Kniffki KD et al., 1981; van Dieen et al., 2003; Murray and Peck 2007; Murray and Lavigne 2014). The data suggest that under goal-directed conditions, influences from higher centers can modify the patterns of change in EMG activity from those proposed by the Pain Adaptation Model or the Vicious Cycle Theory. The effect of pain on motor activity during goal-directed tasks is relevant to our understanding of how orofacial pain patients function in situations where motor performance cannot be compromised, e.g., clearly articulated speech in demanding work situations (teachers, telephonists, public speakers), and chewing unexpectedly hard foods in certain social situations.

Although these collective results of RMS activity did not reveal any significant differences between HS and IS infusions, an individual analysis of RMS EMG activity indicated that, potential individual effects of pain on RMS activity could occur where changes consistent with The *Vicious Cycle Theory* (VCT) or The *Pain Adaptation Model* (PAM) could occur within an individual and pain induced different motor effects between individuals. For example, at the right masseter superior/anterior (RMS/RMA) during the ramp task, 7 participants showed an increase in RMS activity which was consistent with VCT and 10 participants showed a decrease in RMS activity (consistent with the PAM) during HS in comparison with IS infusion. However, during the step task, 7 participants showed a decrease in RMS activity (consistent with VCT) and 10 participants showed increase in activity (consistent with PAM). The findings of current study are consistent with previous findings (Sae-Lee, et al., 2008a; Sae-Lee, et al., 2008b; Hodges, 2011; Hodges & Tucker, 2011; Wiesinger, et al., 2013; Amhameed 2014; Maulina 2013) where individual effects were observed. The literature has detected inter-individual differences not only in responses to pain but also in responses to intervention (Fillingim, 2000).

Our findings are in line with more recent models proposing that motor behaviour is substantially variable between different individuals mediated at multiple levels of the nervous system in the experience or perception of pain (Murray & Peck, 2007; Hodges & Tucker, 2011). These more recent theories (e.g. IPAM) propose that the individual responses to pain results from the interaction between the unique biopsychosocial dimensions of pain (sensory aspects and pain-related cognitions, mood) and the complex

organization of sensorimotor systems. This resultant change in activity might arise because of reorganization in activity at the level of the primary motor cerebral cortex (MI) in order to relieve the pain and thus maintain homeostasis (Murray & Peck 2007).

8. THE INFLUENCE OF PSYCHOLOGICAL VARIABLE ON JAW MOTOR ACTIVITY DURING PAIN

It is generally recognized that psychological factors play an important role in chronic pain (Turner et al., 2001; Lobbezoo, et al., 2002; Fillingim, 2005; Murray GM, Peck CC. 2007; Aggarwal et al., 2010). Recent literature from a number of trigeminal (Lobbezoo, et al., 2002; Brandini, 2011) and spinal studies (Leeuw, et al., 2007; Alschuler, et al., 2008) reports the influence of an interaction between pain and motor activity, and psychological variables such as catastrophizing, depression, stress and/or fear of movement. There is evidence for gender differences in depression, anxiety and stress scores, with females typically reporting more negative responses to pain than males (Edmund Keogh et al., 2001). In another recent study (Akhter, et al., 2014) of experimental pain in masseter muscle, infusion of hypertonic saline resulted in pain intensity, unpleasantness, perceived area, and pain rating indices that were significantly ($P<0.05$) higher in higher pain catastrophizers in comparison with lower catastrophizers. Further, this study showed that there was a slower velocity and higher variability of jaw movements in higher catastrophizing individuals in pain than lower catastrophizing individuals (Akhter et al. 2014).

In order to measure the psychological aspects of chronic as well as experimental pain, there were several self-report measures used in the current study that are well validated

and have been shown to be reliable. All of the participants in the current study had low scores for each of the subscales of depression, anxiety and stress scores (DASS) and the mean scores for each scale were all below 1. Similarly, the pain catastrophizing scores (PCS) showed a total score range of 0 to 33 out of a possible score of 52. Nonetheless, these psychological scores were lower than the average scores obtained from the general population (Lovibond & Lovibond, 1995; Crawford & Henry, 2003). Therefore, no quantitative analysis was performed and a qualitative analysis between catastrophizing score and occurrence of SMU activity during hypertonic saline infusion showed no clear association (see results page 57). A number of reasons could be considered for this absence of an association between psychological measures and measures of motor activity in the present study. A major factor is the inclusion criteria of the current study which was healthy participants. The low scores for the PCS and the DASS is more likely attributed to low psychological distress and supports the notion that all participants were clinically healthy, psychologically well-functioning and were not considered clinically depressed, anxious or distressed which was in accordance with the exclusion criteria of the study (see chapter II page-01).

Another reason could be the difficulty of participants in completely understanding and interpreting the questionnaires specially those who had non-English speaking background. Although experimental pain models in healthy individuals can provide similar sensory and discriminative aspects of the experience of clinical pain, they are unable to replicate closely the emotions and cognitions present in chronic pain patients (Lobbezoo, et al., 2006). The findings of the current study are consistent with one of our recent studies of experimental pain in the anterior temporalis and masseter where psychological distress

also showed no significant correlation with the RMS EMG activity during different jaw movement tasks during hypertonic and isotonic saline infusion (Amhamed, 2014). In addition, a clinical musculoskeletal pain study revealed no relationship between psychological distress and physical functioning (Helmus et al., 2012). Therefore, our findings lead to the need for future research to further investigate the influence of psychological factors on the objective measures of jaw functions in the clinical pain patients using appropriate psychological measures.

9. OVERAL SUMMARY OF EFFECTS OF PAIN ON EMG ACTIVITY

We have refined the experimental muscle pain paradigm which allows us to simultaneously record the EMG activity from 2 different sites within the masseter muscle during standardized isometric jaw tasks. We studied the effects of experimental jaw muscle pain on isometric jaw tasks by assessing single motor unit activity and jaw force generation in different experimental recording sessions i.e., baseline control, hypertonic saline infusion and isotonic saline infusion. The infusion of 5% hypertonic saline into the deep central region of the right masseter muscle provoked pain, some of the features of which were similar to chronic orofacial pain. With this standard model, pain intensity levels of 3-6/10 VAS were relatively constant throughout the infusion period and all jaw tasks were performed in a single sitting on a single experimental day.

Muscle activity during each of the tasks from each of the recording sites in the right masseter muscle under pain and control conditions was established by discriminating individual single motor units (SMUs) from the intramuscular EMG recordings from the

masseter muscle. The findings of the present study were generally inconsistent with the proposals of the vicious cycle theory and the pain adaptation model which propose a uniform effect of pain on muscle activity throughout a muscle. Further, the pattern of occurrence of motor units was compared between different recording sessions and we found that during jaw muscle pain in comparison with isotonic saline infusion, the pattern of activity could vary while performing the same isometric jaw tasks. For example, across both sites, there were 6 (12% of total of 50 SMUs) newly recruited single motor units during HS infusion that were not present during IS infusion. A qualitative comparison of these patterns of activity at the two different sites within the masseter showed that some patterns of occurrence of SMUs were consistent with the vicious cycle theory or the pain adaptation model, while some patterns were not consistent with either model at the two different sites within the masseter.

An important finding from the present study was how the jaw motor system functions under pain in order to perform the particular jaw motor task. The results showed that experimental masseter muscle induced by 5% hypertonic saline does not alter the ability of individuals to perform isometric jaw-closing tasks, experimental masseter muscle pain led to changes in the recruitment patterns, but not the firing rates or thresholds of single motor units. The data provide support for more recent models proposing a re-organization of the recruitment strategy adopted by the brain in the control of motor units in the presence of pain. Our findings suggest that the sensorimotor system employs an altered motor unit recruitment strategy at different sites within a heterogeneous muscle in order to perform particular jaw motor tasks in pain in comparison with control. Further, the

inter-individual variability in hypertonic-saline-induced intramuscular EMG effects on the standardized isometric jaw-closing tasks at two different sites of muscles was also observed in the present study. The findings of the present study open a new window for understanding the effects of pain on other jaw muscles and may have implications for the development of future studies of pain effects in other muscles of the body. These findings may also have clinical significance with regard to the variability in clinical manifestations between orofacial pain patients.

9.1 Methodological Limitations

- The masseter muscle is functionally heterogeneous (Munro and Griffin, 1970; Vitti and Basmojian, 1977; Van Eijden, 1990; Blanskma et al., 1992). Despite the fact that the current study has allowed us to accurately observe the effect of experimental muscle pain on single motor unit activity at two different sites of the masseter muscles, we were not able to study all compartments involved in the required jaw task and it might be possible that other parts of the masseter muscle not studied here might show markedly different patterns of activity during hypertonic saline infusion than from the two recording sites employed in the present study. A more comprehensive study that involves more compartments will provide us with more information as to the effects of pain on motor activity. Further, it is unclear the fibre types recorded at the different sites.
- The data of the first 3 participants were recorded from anterior and posterior locations but the SMU data were combined with the remaining participants' data, as described in the methods. This is a limitation of the analysis although not a major issue as the SMU analysis was a within-subject design.

- Despite the fact that the current study has allowed us to accurately observe the effect of experimental muscle pain on single motor unit activity at two different sites of the masseter muscles, we were not able to study the effect of pain on SMU activity within contralateral muscles. A more comprehensive study that involves both agonist as well as antagonist muscle will provide us with more information as to the effects of pain on motor activity.
- Another possible limitation of the present study is the small sample size which limits the generalizability of findings regarding pain-motor interaction and for assessing psychological effects. The chances of a type I error are increased by the number of analyses performed in proportion to the size of the sample.
- The type of experimental muscle pain used in this study was acute tonic jaw muscle pain induced by hypertonic saline and therefore the findings are limited in their implications for understanding chronic pain.
- A possible limitation of this study is inter-individual changes in the placement of the ball on the sensor during the installation of the sensor according to the relationship of the location between upper and lower jaw and hence, this study discusses the force only within participants and does not refer to the comparison between the participants.

9.2 Future Research Directions

- As the results in the current study were obtained from two specific sites of masseter (RMS/RMA and RMI/RMP), the suggested de-recruitment behaviour cannot be automatically assumed for other regions or for the whole muscle. This

limitation also applies to the individual force values and their effect on recruitment modalities although it has been expected that the characteristics of neuromuscular behaviour in other muscle regions, and possibly also at distinct force levels are similar. Although the present experimental pain study has extended our knowledge into the effect of pain on motor unit activity, future studies are needed to test this effect in different compartments of masseter muscle and also with different tasks.

- The functional reorganization of jaw motor function observed in the present study needs to be examined under situations where tonic pain is induced simultaneously in the agonist and antagonist muscle pair or presumably in more than two muscles (right and left).
- The present study only explored the effects of noxious masseter muscle stimulation on the activity of motor units within the masseter. Future research can include studying the effects of hypertonic saline on the activity of single motor units of other muscles while investigating the influence of psychological factors on the objective measures of jaw functions. These studies are currently underway (Moura, Sandoval, et al.).
- A more detailed method of recording single motor unit activity in orofacial musculature is by using patch equipment as used by Tucker and Hodges in limb and trunk which will give enhanced detail of overall activity in the experimental muscles (Tucker and Hodges, 2009).

- Future studies could utilize a 3-dimensional force transducer to determine if pain evokes subtle changes in force direction, as has recently been demonstrated in the leg muscle system (Tucker and Hodges, 2010).
- Translated versions of questionnaires into different languages should be available in research labs which will help enabling the participants having non English backgrounds to better understand the questionnaires.
- There may be a dose-response relation but this was not examined in the present study but is an avenue for further study.
- Finally, our findings are entirely consistent with recent findings in the spinal literature (Tucker et al., 2009). Nonetheless, the current study provides a baseline for future studies. Since the jaw motor system is complex and mechanically redundant and the performance of jaw motor task involved number of different muscle, future researches are motivated to govern the effects of hypertonic saline on activity of single motor units of 2 or more muscles while investigating the influence of psychological factors on the objective measures of jaw functions using appropriate psychological measures. In addition age and gender should be evaluated in future studies to see the difference in pain perception and muscle activity changes.
- DOMS could be possibly used as another model of inducing muscle pain compared to hypertonic saline infusion which could be clinically more relevant to TMD and could provide more insight into the pathophysiology of TMD.

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