REORGANIZATION OF JAW MUSCLE ACTIVITY DURING EXPERIMENTAL JAW MUSCLE PAIN

by

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DECLARATION

I hereby declare that this dissertation is a result of the work of the author, excepted where the correct acknowledgements is otherwise mentioned. The work has not been previously accepted to any other University or Institute forward of any degree or diploma. It is also declared that the presented information is true and substance for the fulfilment of the requirement of the Doctor degree by the Research degree program at the Faculty of Dentistry, The University of Sydney and was carried out at the Jaw Function and Orofacial Pain Research Unit, Westmead Hospital under the supervision of Professor Greg Murray.

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ABBREVIATIONS

ANOVA: Repeated-measures analysis of variance

CBT - Cognitive-behavioural therapy.

CGRP - Calcitonin gene–related peptide

CNS - Central nervous system

DASS21 - Depression, Anxiety and Stress Scales 21

DC/TMD – The Diagnostic Criteria for Temporomandibular Disorders

DRG - Dorsal root ganglia

EMG – Electromyography / electromyographic

FG – Fast glycolytic

FOG - Fast oxidative glycolytic

FPL - flexor pollicis longus

FPS - 4-point scale

FPS-R - Faces Pain Scale–Revised

FRs - Firing rates

H SR - Slow ramp jaw closing task during hypertonic saline infusion.

IASP – International Association for the Study of Pain

IBS - Irritable bowel syndrome

ICMS - Intracortical microstimulation

IPAM – The Integrated Pain Adaptation Model

IPT - Iowa Pain Thermometer

I SR - Slow ramp jaw closing task during isotonic saline infusion.

MHC - Myosin heavy-chain

MI - Primary motor cortex

MPQ - McGill Pain Questionnaire

M.U.A.P. - Motor unit action potentials

MUAPTs - Motor unit action potential trains

MVBF - Maximum voluntary bite force

NK-1 - Neurokinin-1

NMDA - N- methyl- d –aspartate

NRS - Numeric rating scale

NS - Nociceptive specific

PAS - Periodic acid Schiff

OFP – Orofacial pain

PAM – The Pain Adaptation Model

PNS - The peripheral nervous system

PCS - Pain Catastrophizing Scale

RMI/RMP – Right masseter inferior/posterior site

RMS/RMA – Right masseter superior/anterior site

RDC/TMD - The Research Diagnostic Criteria for Temporomandibular Disorders

RMS – Root means square

sEMG - Surface electromyography

SI- Primary somatosensory cortex

SMU - Single motor unit

SO - Slow oxidative

STN - Solitary tract nucleus

TMD - Temporomandibular disorder

TMDs – Temporomandibular disorders

TMJ - Temporomandibular joints

trkB - Tyrosine kinase B

TRPV1 - Transient receptor potential

VAS - Visual analogue scale

VBSNC - Trigeminal brainstem sensory nuclear complex

VCT - The Vicious Cycle Theory

VDS- Verbal descriptor scale

VRS - A verbal rating scale

WDR - Wide dynamic range

PRESENTATIONS:

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

Oral Presentation:

Polyana Moura, Isbel Sandoval, Terry Whittle, Greg Murray. Reorganization of jaw muscle activity during experimental jaw muscle pain - Faculty Research Day, Faculty of Dentistry, The University of Sydney, Westmead, NSW, Australia, September, 2017 – With honorable mention (2nd place in R. G. Schamschula Prize for Higher Degree by Research presentations).

Poster Presentation:

Polyana Moura, Isbel Sandoval, Terry Whittle, Greg Murray. Reorganization of jaw muscle activity during experimental jaw muscle pain - Hospital week, Faculty of Dentistry, The University of Sydney, Westmead, NSW, Australia, August, 2017.

ABSTRACT:

Background and Aims: Temporomandibular disorders are clinical conditions that often involve pain in the masticatory muscles, the temporomandibular jaw joint and/or associated structures. The association between muscle pain and muscle activity is often explained by uniform increases or decreases in motor unit activity throughout a muscle but recent evidence suggests more complex changes within a painful muscle. The general aim of this study was to determine if experimentally induced masseter muscle pain modifies temporalis muscle activity.

Methods: 20 healthy participants received experimental pain through hypertonic saline (5% NaCl) infusion into the right masseter; pain intensity was maintained at 40-60/100 mm on a visual analogue scale (VAS). Standardized biting tasks were performed with an intraoral force transducer while single motor unit (SMU) activity was recorded from 2 intramuscular electrodes (right masseter and right temporalis). The tasks were repeated in 4 blocks: baseline 1, hypertonic saline infusion, isotonic saline infusion, baseline 2. Each block had 3 isometric biting tasks: a slow and a fast ramp jaw closing task and a 2 step-levels jaw closing task (2 force levels: step 1 and step 2).

Results: 83 SMUs were discriminated from the temporalis and 58 from the masseter muscle. This study demonstrated that induced muscle pain in the right masseter can be associated with the activation of new SMUs and the silencing of other single motor units in the painful masseter muscle as well as in the right temporalis muscle, which did not receive noxious stimulation with the hypertonic saline. No differences between pain and no pain trials were found in thresholds and firing rates of SMUs from the temporalis muscle.

Discussion and conclusion: The present findings are consistent with previous findings from the limb (Hodges and Tucker 2011; Tucker et al. 2009) and rather than supporting uniform increases or decreases in motor unit activity throughout a muscle, suggest that there is a reorganization of motor unit activity across the entire jaw motor system in experimental pain.

RATIONALE FOR THE THESIS:

Temporomandibular disorders (TMDs) are clinical conditions that often involve pain in the masticatory muscles, the temporomandibular or jaw joint and/or associated structures. They impose significant personal and economic burdens on ~5% of the population (Blyth et al. 2001; Sessle 2000). The cause is only partly understood and some current treatments are based on little scientific evidence.

For many years, there was thought to be a Vicious Cycle between pain and muscle activity that perpetuated the pain. This theory and a more recent theory, the Pain Adaptation Model, however are not strongly supported by the literature. For example, both theories propose uniform increases or decreases in activity throughout a painful muscle but recent evidence in the spinal motor system suggests that there are likely to be complex changes of activity within a painful muscle (these changes indicate a re-organization of activity) instead of uniform changes in activity throughout the painful muscle.

The principal investigators and research staff in our research unit have recently demonstrated this re-organization within one of the jaw muscles, the masseter muscle (Malik 2016; Minami et al. 2013). The new data suggest the need to re-assess management strategies based on models that propose uniform effects of pain on muscle activity. It is well known that TMDs are frequently characterized by pain in a number of jaw muscles. It is unclear however whether this pain-induced reorganization of activity that has been demonstrated within one jaw muscle also occurs in other jaw muscles and whether indeed this reorganization in other jaw muscles could contribute to spread of the pain condition. This information would assist in understanding the pathophysiology of TMDs.

Another issue with these earlier theories is that they do not take psychological factors into consideration and yet psychological factors are known to be important in TMDs. Recent studies from our group (Akhter et al. 2014) have provided evidence that one psychological factor (namely, catastrophizing) may be a factor that influences the effects of pain on jaw muscle activity. The question arises whether psychological factors might influence the reorganization that appears to be occurring within the jaw muscles during pain, and thereby possibly influence symptoms.

The present study has used electromyographic (EMG) recordings from the jaw muscles to assess whether complex changes in activity can occur throughout the jaw muscles during experimentally induced jaw muscle pain. We also employed questionaries, namely, the Depression, Anxiety and Stress Scales (DASS) (Lovibond and Lovibond 1995a), the McGill Pain Questionnaire (MPQ) (Melzack 1975) and the Pain Catastrophizing Scale (PCS) (Sullivan MJL 1995), to look for associations between any changes in muscle activity patterns and one or more of these psychological factors.

The information obtained from these studies may help to improve our understanding of the effects of pain on jaw muscle activity and thereby may have implications for understanding changes in jaw muscle activity in TMD and the spread of symptoms.

1 REVIEW OF THE LITERATURE

1.1 Jaw motor system

The jaw motor system is anatomically and physiologically a very complex structure consisting of the maxillae and mandible, teeth, periodontium, tongue, temporomandibular joints and orofacial muscles. Because of its complexity, the jaw system is able to generate large compressive forces during mastication and also to achieve very fine motor control with precise positioning of the upper and lower teeth whenever it is required (Peck et al. 2010). Given the frequent demands imposed on the jaw motor system from, for example, the need for clearly articulated speech and efficient masticatory activities, then it may be not surprising that this highly complex motor system can develop pain and/or dysfunction.

1.2 Pain and temporomandibular disorders (TMDs)

The development of a universally accepted definition of pain and related concepts was indicated by John J Bonica as one of the main goals of the then recently formed International Association for the Study of Pain (IASP) (Classification of chronic pain. Descriptions of chronic pain syndromes and definitions of pain terms. Prepared by the international association for the study of pain, subcommittee on taxonomy 1986) (Raffaeli and Arnaudo 2017).

Therefore, pain has been defined, according to IASP as *"an unpleasant, sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage"* (Lipton 1991). This definition includes the sensory aspect of pain as well as the emotional and interpretive or cognitive aspects.

In fact, the attempt to understand pain represents one of the oldest challenges in the history of medicine (Raffaeli and Arnaudo 2017). It leads patients to seek medical relief, perhaps more than any other one symptom of disease (Wagner 1906).

Orofacial pain refers to pain in the face and/or oral cavity. Studying one population dataset from the UK Biobank between 2006 and 2010, researchers reported an overall prevalence of self-reported facial pain as 1.9% (women 2.4%, men 1.2%) of which 48% was chronic pain (Macfarlane et al. 2014). Another study, analysing the prevalence of orofacial pain (OFP) among young adults (30-31 years old), reported the prevalence of OFP as high as 23% (Macfarlane et al. 2009).

Despite the fact that it is very prevalent in the population, pain also includes different types, for example, associated with pulpal and periodontal processes, sinusitis, trigeminal neuralgia, and pain in any of the masticatory muscles or temporomandibular joints (TMJ) (Conti et al. 2012).

Temporomandibular disorders (TMDs) are clinical conditions that often involve pain in the masticatory muscles, the temporomandibular jaw joint and/or associated

structures. The most common symptom is pain, but often patients with TMD present other symptoms such as limited or asymmetric mandibular movements and/or joint sounds (Yap et al. 2002) and which vary in severity from case to case.

Temporomandibular disorders are a very common problem affecting up to 33% of individuals within their lifetime (Wright and North 2009). However, this prevalence can vary from study to study as the number of patients suffering from TMD seems to be increasing worldwide. Temporomandibular disorders are in fact one of the most common chronic pain conditions, along with headaches and back pain (Ghurye and McMillan 2015).

Epidemiological studies show that nearly 10% to 15% of the general population has some kind of TMD, and from those, around 5% require treatment (Tournavitis et al. 2017). They impose significant personal and economic burdens on ~5% of the population (Blyth et al. 2001; Sessle 2000) and also have working related impacts. Treatments often require a multi-disciplinary approach and they represent a significant health-care cost with non-surgical treatments often costing over $1,000 per episode.

In general, TMDs are more prevalent in women during their reproductive years than men and post-menopausal women. Some authors have suggested that TMDs can have estrogen as a risk factor as it could contribute to enhanced central sensitization processes (see section 1.4.10 below) and which might predispose to the development of painful TMD (Ribeiro-Dasilva et al. 2017). In support of this idea, another study showed no difference in the prevalence of TMD between boys and girls during the pre-puberty phase of their lives (Ghurye and McMillan 2015).

Nowadays, there are a large number of therapeutic options that can help with the elimination or reduction of the symptoms in muscles and jaw joints. For example, some therapeutic options include the occlusal oral plates (Restrepo et al. 2011), physical therapy (Morell 2016; Tuncer et al. 2013), cognitive-behavioural therapy (CBT) (Dura-Ferrandis et al. 2017), acupuncture (Fernandes et al. 2017), stress reduction strategies (Reissmann et al. 2012), physical exercises (Haggman-Henrikson et al. 2017b), attention to the improvement of sleep quality (Babiec 2017), pharmacotherapy (Haggman-Henrikson et al. 2017a), counselling and self-care management (Henien and Sproat 2017; Nilsson and Willman 2016), laser therapy (Demirkol et al. 2017) or even a combination of two or more of these options depending on the case and the recommendation of the dentist. However it has been reported that there is a lack of therapeutic confidence in the treatment of pain related to TMD among dentists (Kakudate et al. 2017).

The stabilization appliance using a flat occlusal splint made of hard acrylic or polycarbonate material is among the most popular current treatment worldwide (Altok et al. 2016; Shukla and Muthusekhar 2016). Yet, insufficient evidence for its effects on musculature is found on the literature (Al-Ani et al. 2004; Dahlstrom et al. 1982) and further studies are recommended in regards to occlusal stabilization as a TMD treatment (Kuzmanovic Pficer et al. 2017).

Although it has been widely discussed by researchers and clinicians, the cause and pathophysiology of TMD remains unclear and many current treatments are based on little scientific evidence, and there is a significant placebo effect. Therefore, patients

with TMD can go from one clinician to the next in a desperate attempt to obtain symptom relief. Besides, not much is known about the reason and mechanisms whereby acute TMD episodes became chronic TMD.

Chronic pain is usually defined as pain lasting more than 3 months and almost certainly has some element of central sensitization (Crofford 2015; Gil-Martinez et al. 2016). It is a complex sensory and emotional experience that varies widely between people depending on the context and meaning of the pain and the psychological state of the person (Bushnell et al. 2013). It can be really disabling with a negative impact on people's quality of life. The value of this definition is its ability to describe all the conditions that can be defined as chronic pain even if it does not refer to the impairment brought about by the pain, the presence of specific symptoms, and the supposed etiologic framework. This is also because chronic pain is a term employed to define several diverse conditions whose common feature is the presence of persistent pain (Raffaeli and Arnaudo 2017).

In this context, TMD may also be associated with other problems of general health, depression and anxiety, or psychological disabilities that affect the patient's quality of life or even elevated levels of suicidal ideation (Bertoli and de Leeuw 2016; Stohler 1999). In fact, it is frequently associated with physical symptoms of other chronic pain disorders and comorbidities, as generalised muscle and joint pain (Beiter et al. 2015; Moreno-Fernandez et al. 2017), or irritable bowel syndrome (IBS) (Gallotta et al. 2017), for example. TMD actually shares similarities with other chronic pain conditions such as chronic tension-type headache or migraine, low back pain, and fibromyalgia in physiopathologic mechanisms (Sessle 2009). Also, one study found no differences

between TMD patients and non-TMD patients with a chronic pain disorder in terms of the use of medicines, levels of depression, anxiety, somatization, hostility, psychoticism, behavior in their social surroundings, ratings of problems with work, family, self-esteem, or impulses for suicide (McKinney et al. 1990).

1.3 TMD and diagnoses

The diagnosis of TMD is complex. Most current research recognizes that TMD is not caused by a single factor but it is multifactorial. Temporomandibular Disorders are complex disorders that are best viewed from a biopsychosocial perspective, that is, they exhibit a range of different physical signs and symptoms, as well as changes in behaviours, and emotional and social interactions (Slade et al. 2016; Svensson and Kumar 2016). This has led to acceptance of a multifactorial ethology of TMD pain.

There are several diagnostic systems proposed for TMD.

The Research Diagnostic Criteria for Temporomandibular Disorders (RDC/TMD) was published in 1992 and it was the first classification that incorporated the biopsychosocial pain model. It has been translated into 22 languages with a Dual-axis system: clinical conditions (Axis I) with the purpose of finding possible abnormalities of structures and functions of the masticatory muscles and temporomandibular joints, and pain-related disability and psychological status (Axis II) (Dworkin and LeResche 1992).

The RDC/TMD has been the most extensively used diagnostic protocol for TMD research since its publication. The intent was to provide simultaneously a physical diagnosis and to identify other relevant aspects of the patient that could influence the expression of TMD and maybe help with the management of their pain (Schiffman et al. 2014).

A series of studies has been done since its publication to verify its diagnostic validity and to identify some points of conflict. In this context, the Diagnostic Criteria for TMD (DC/TMD) is the revised version of the RDC/TMD and evaluations indicate that it is reliable and valid. This diagnostic protocol cover the most frequent kinds of TMD, such as disorders related with pain (e.g., myalgia, TMD-related headache and arthralgia) and disorders linked to the TMJ (e.g., disc displacements and degenerative disease) (List and Jensen 2017).

While the new DC/TMD protocol promises to be an important tool for future clinical diagnosis and management as well as clinical research projects, a limitation of the new DC/TMD is that it only partly addresses TMD mechanisms and etiologies. Furthermore, it is now recognized that TMD are a heterogenous group with manifestations well beyond the signs and symptoms associated with the current Axis I diagnoses. More comprehensive medical assessment of comorbid physical disorders and biobehavioral status with expansion of Axis II risk determinants for TMD will facilitate the identification of subpopulations of patients based on underlying pathophysiological mechanisms (Schiffman, Eric et al 2014).

1.4 Single Motor Unit (SMU) and electromyography (EMG) activity

Knowledge of the anatomy and physiology of the motor mechanism that is required to produce muscle contractility in human beings and other animals is essential in order to understand the variety of disturbances that can occur in human motion and locomotion. Therefore, electromyography is the study of the electrical activity of the muscle (Pruzansky 1952) and is used for studying muscular functioning – See figure 1-1.

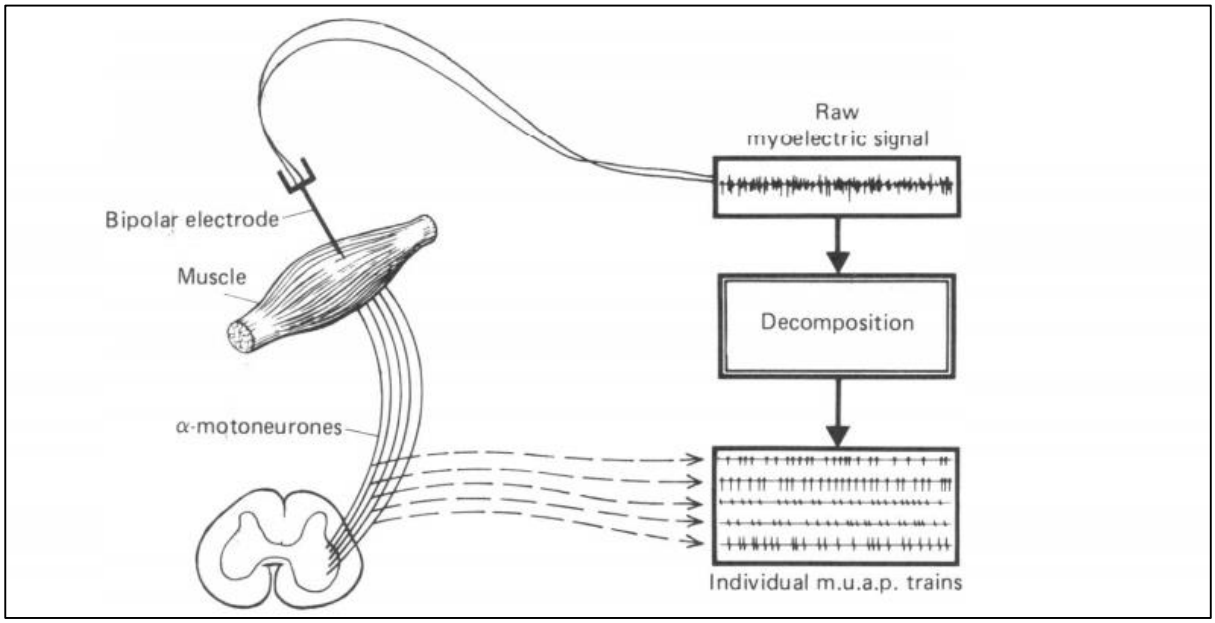


Figure 1-1: Schematic illustration of the origination, myoelectric recording and subsequent decomposition of five superimposed motor-unit action potential. M.u.a.p.: motor unit action potentials From: (De Luca et al. 1982).

Consequently, EMG analysis might be helpful to elucidate the normal function of the masticatory muscles as well as how the muscles adapt in patients with TMD Svensson and Graven-Nielsen 2000.

Movement is accomplished by the controlled activation of motor unit populations. The motor unit is mostly under the control of the central nervous system (CNS). The motor unit is the final common pathway whereby converging sensory and descending neural inputs are translated into forces to generate movement. It is therefore a neuromechanical transducer.

1.4.1 Definition of a motor unit

The motor unit (Figure 1-2) consists of two components:

(i) One α-motoneurone and

(ii) The muscle fibres innervated by that motoneurone (Heckman and Enoka 2012).

Each muscle fibre is innervated by only one motoneurone, and each motoneurone can innervate between ten and thousands of muscle fibres. The muscles that act on the largest body masses have motor units that contain more muscle fibres, whereas smaller muscles contain fewer muscle fibres in each motor unit (Buchthal and Schmalbruch 1980). Jaw muscle motor units contain several hundred muscle fibres (Lenman and Ritchie 1987).

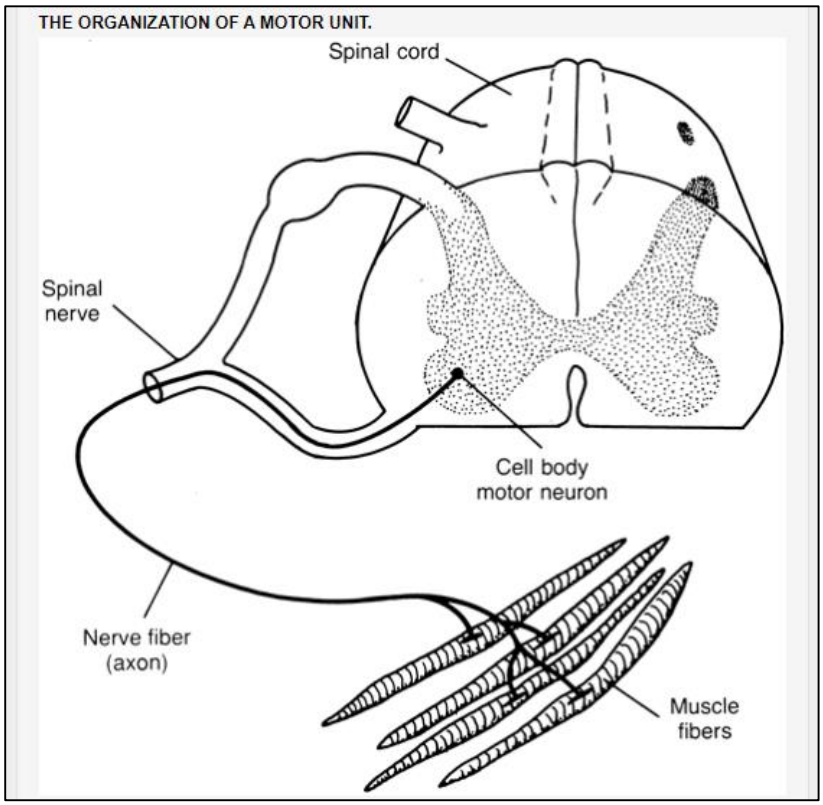


Figure 1-2: The organization of a motor unit from the spinal motor system. The motor unit consists of a motoneurone (motor neuron in the figure) and all the muscle fibres it innervates. From (Hamilton et al. 2011).

1.4.2 Fibre types

Skeletal muscles are composed of numerous fibres that range from 10 to 80 micrometers in diameter. These fibres contain myofibrils, which can be defined as the real force generators. Each myofibril consists of sarcomeres, the final functional units of the muscle contraction. The sarcomeres, in turn, contain mainly thick (myosin) and thin (actin) filaments. Nonetheless, there are also other proteins called troponin and tropomyosin –See figure 1-3. The interaction between all these filaments is the cornerstone for muscle contraction (Bottinelli and Reggiani 2000).

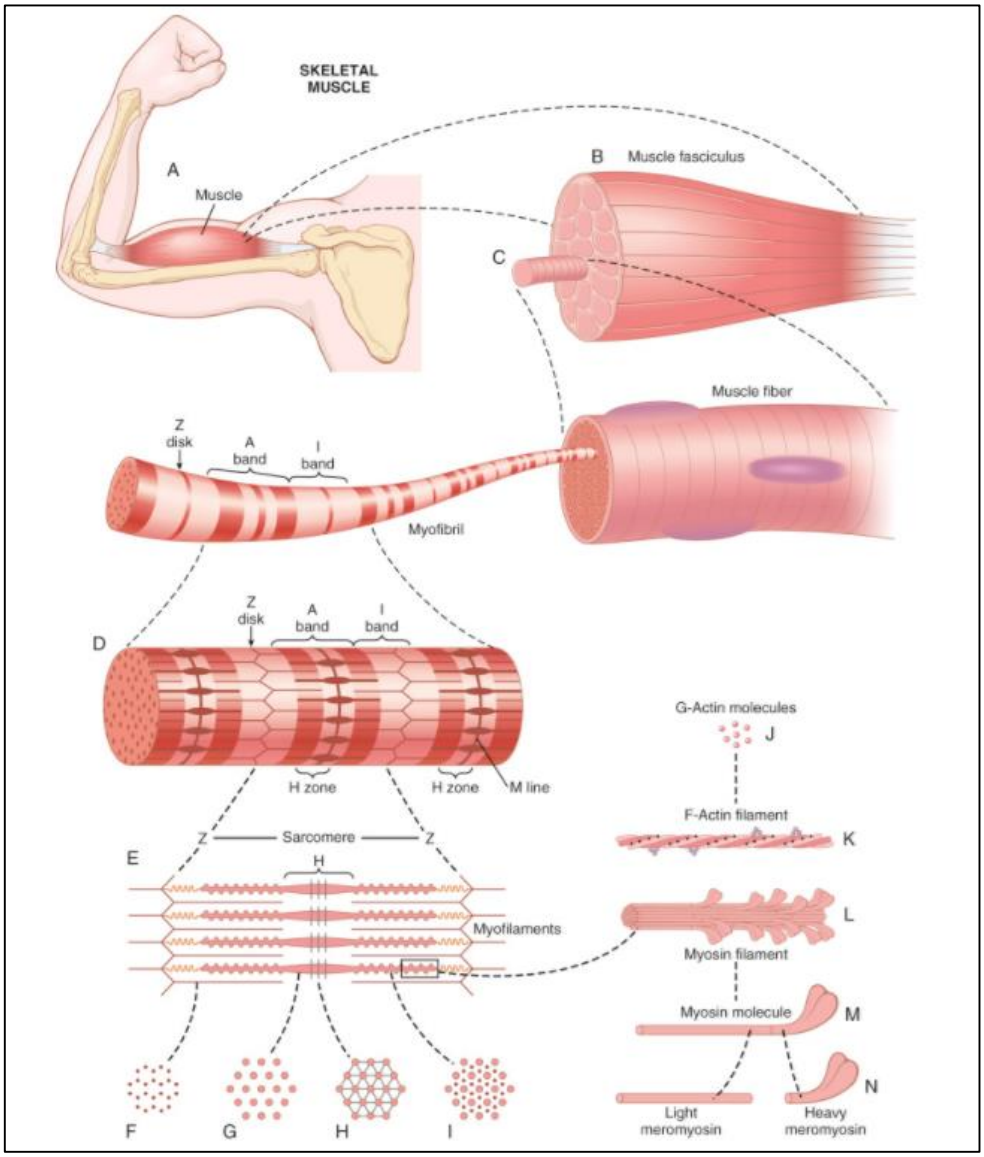


Figure 1-3: Organization of skeletal muscle (A and B), from the gross to the molecular level. Each muscle fibre (C) can contain from several hundred up to several thousand myofibrils (D). The sarcomere (E) is the part of the myofibril between two successive Z discs. (F), (G), (H), and (I) are cross sections at the levels indicated. Each myofibril is composed of around 1500 adjacent myosin and 3000 actin filaments, which are large polymerized protein molecules responsible for the actual muscle contraction (J), (K), (L), (M), (N). From: (Hall 2016).

Scientists have been aware that skeletal muscles can be distinguished on the basis of their colour as red or white and their contractile properties as fast and slow since the first half of the 19th century (Schiaffino and Reggiani 2011).

Initially, three types of muscle fibres were histochemically distinguished exclusively on the basis of qualitative differences in their actomyosin ATPase which could distinguish them between type I (slow) and type II (fast) fibres (Guth and Samaha 1970). Later on, type II fibres were subdivided into IIA and IIB and some new fibre types (IIX, IIC and IM) were also identified by this method (Staron and Pette 1986).

Also, based on histochemical techniques using both mitochondrial enzyme and myosin adenosine triphosphatase (ATPase) activities to differentiate fibres, muscle fibres can be classified as slow oxidative (SO), fast oxidative glycolytic (FOG), and fast glycolytic (FG) (Delp and Duan 1996; Pette and Staron 1990).

In addition, the structural and functional properties of the fibres, which are generally referred to as fibre phenotype, can change in response to hormonal and neural influences, nerve-activity being a major determinant of the fibre type profile. This property is defined muscle plasticity or malleability (Schiaffino and Reggiani 2011).

The association of different muscle fibre types with motor neurones of different sizes is the basis of an elegantly simple mechanism for grading the force of muscle contraction.

1.4.3 Small x large motor units

Motor units may vary in size. The size of a motor unit in a muscle is defined by the innervation ratio, which is the ratio between the number of muscle fibres in a muscle and the number of supplying motoneurone axons. Small motor units have small-diameter axons typically innervating slow muscle fibres that are resistant to fatigue. Those motor units play one important role in activities that require sustained muscular contraction, as, for example, in the maintenance of an upright posture. Large motor units have, on the other hand, large-diameter axons that typically innervate faster muscle fibres that are more fatigable and play an important role in brief exertions that require large forces, as for example running or jumping. Lastly, there is also an intermediate-size motor unit called fast intermediate fatigable or fast fatigue-resistant –See figure 1-4 (Burke 1967; Burke et al. 1970; Burke et al. 1973; Purves D 2001).

The size of a motor-unit is especially important in determining the magnitude of the motor-unit force and the amplitude of the motor-unit action potential (Goldberg and Derfler 1977).

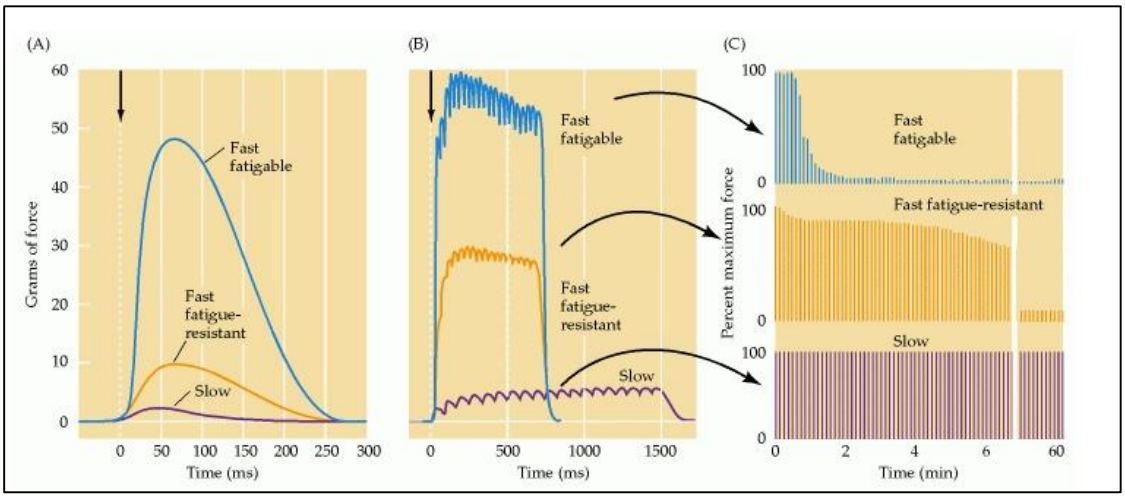


Figure 1-4: Three different types of motor units and their force and fatigability. (A), (B) and (C) shows the change in muscle tension due to (A): A single motor neurone action potential. (B): Repetitive stimulation of the motor neurones. (C): Repeated stimulation at a level that evokes maximum tension. The ordinate represents the force generated by each stimulus with the different fatigue rates. From: (Purves D 2001).

1.4.4 Motor unit variability

Most information on the physiological properties of single motor units comes from animal experiments involving stimulation of motoneurones directly by insertion of electrodes into the motor nucleus supplying the limb muscles (Freund 1983).

SMUs can vary in morphology (innervation ratio, cross-sectional area, geometric distribution of muscle fibres) and in physiological and biochemical properties (force output, contraction velocity, resistance to fatigue, oxidative and glycolytic capacities, ATPase activities, and myosin heavy-chain (MyHC) isoform contents) (van Eijden and Turkawski 2001). Masticatory muscles contain, therefore, a large variety of motor units

with different physiological and morphological properties (Turkawski and van Eijden 2000).

Many differences can be found between and within the jaw muscles. In fact, a comparison of jaw opening muscles with jaw closing muscles shows that jaw openers are simpler with respect to activation, architecture and fibre type composition than the jaw closing muscles (Tsuruyama et al. 2002). In a study analyzing fibre type compositions and fibre cross-sectional areas of masticatory muscles in eight cadavers (Korfage et al. 2000), it was demonstrated that the temporalis, masseter and pterygoid muscles could be characterized by a relatively large number of fibres containing more than one MyHC isoform (hybrid fibres) with a large number of fibres expressing MyHC-I, MyHC-fetal and MyHC-cardiac alpha. Besides, in these muscles type I fibres had larger cross-sectional areas than type II fibres. In the meantime, the mylohyoid, geniohyoid and digastric muscles were characterized by less fibres expressing MyHC-I, MyHC-fetal, and MyHC-cardiac alpha, and by more fibres expressing MyHC-IIA; the cross-sectional areas of type I and type II fibres in these muscles did not differ significantly. Furthermore, jaw closer muscles contain 40% of hybrid muscle fibres as opposed to only 10% of these fibres in jaw opener muscles (Korfage et al. 2001).

1.4.5 Orderly recruitment of motor units

Adrian and Bronk (Adrian and Bronk 1929) initially discussed that increases in force were achieved by the recruitment of additional motor units and an increase in discharge rate of motor units that had already been recruited. Derek Denny-Brown (1901–1981) and Joseph Pennybacker (1907–1982) reported later, in 1938, that "*a particular voluntary movement appears to begin with discharge of the same motor unit. More*

intense contraction is secured by the addition of more and more units added in a particular sequence. This 'recruitment' of motor units into willed contraction is identical to that occurring in certain reflexes. The early motor units in normal graded voluntary contraction are always in our experience small ones. The larger and more powerful units, each controlling many more muscle fibres, enter contraction late" (Denny-Brown 1938).

Elwood Henneman et al. (Henneman 1957; Henneman et al. 1965) finally proposed that, with increasing levels of motor activation, motor units would be recruited in order, starting from the smallest to the largest and this is termed the Size Principle of Motor Unit activation. On the basis of this, the recruitment of the smallest, most fatigue-resistant motor units would be recruited in tasks requiring fine motor control over long periods of time, while the larger motor units would be required for brief bursts of high force production – See figure 1-5 (Mendell 2005).

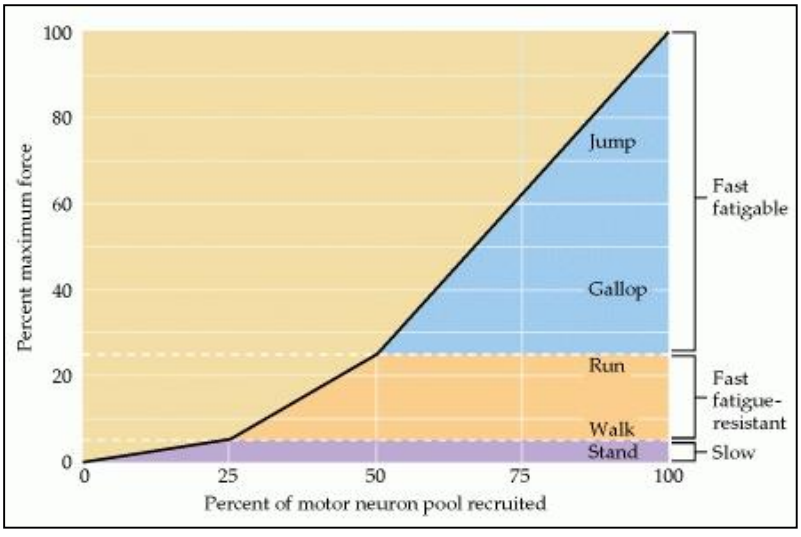


Figure 1-5: The recruitment of motor neurones in the cat medial gastrocnemius muscle under different behavioural conditions. Slow motor units, for instance, provide the tension necessary for standing. Fast fatigue-resistant (FR) units, in turn, provide additional force required for activities such as walking and

light running. Lastly, fast fatigable units are recruited for the most demanding activities. From: (Purves D 2001). -After (Walmsley et al. 1978).

1.4.6 Motor unit territories

In humans the size of the individual motor unit is investigated by electrophysical delineation of the territory occupied by the fibres of a motor unit. Fibres are identified as belonging to the same motor unit when their potentials have identical time relationships irrespective of the frequency of discharge (Buchthal and Schmalbruch 1980). In this technique, an electrode is moved through the territory of an active motor unit. The length of the path over which motor unit activity is registered is used as an estimation of motor unit width (van Eijden and Turkawski 2001).

In animal muscles, motor unit territories can be mapped with the glycogen-depletion method that was originally described by Edstrom and Kugelberg (1968). In this method, a neurone is stimulated until the muscle fibres it innervates are depleted of glycogen. These depleted fibres can then be visualized in a muscle histological section, where they are the only ones that are not stained by the periodic acid Schiff (PAS) method (van Eijden and Turkawski 2001).

Muscle fibres of motor units are intermixed with each other and are restricted to a particular region of the muscle, named the motor unit territory. Within the territory of a motor unit, motor units of human limb muscles have an average territory of 5-10 mm in diameter; this allows space for the fibres of 15-30 other motor units to be

intermingled (Buchthal and Schmalbruch 1980). The distance between fibres of a particular motor unit varies from zero (fibres in contact) to hundreds of micrometres.

In two muscles with equal average territory of the motor units (e.g., the brachial biceps and the extensor digitorum communis), the maximum amplitude of the motor-unit potential is highest in the muscle with the greatest number of fibres per motor units (Buchthal and Schmalbruch 1980).

Motor unit territories in masticatory muscles seem to be smaller than territories in limb muscles, and this would indicate a more localized organization of motor control in masticatory muscles. Motor unit cross-sectional areas show a wide range of values, which explains the large variability of motor unit force output. The proportion of motor unit muscle fibres containing more than one MHC isoform is considerably larger in masticatory muscles than in limb and trunk muscles. In fact, in trunk and limb muscles, this phenomenon is primarily observed in elderly subjects (Andersen et al. 1999). Hence, in masticatory muscles, a finer gradation of force and contraction speeds appears when compared to limb and trunk muscles (van Eijden and Turkawski 2001).

The presence of localized motor unit territories and task-specific motor unit activity facilitates differential control of separate muscle portions. This gives the masticatory muscles the capacity of producing a large diversity of mechanical actions (van Eijden and Turkawski 2001).

1.4.7 Motor units and the contraction of the muscles

The ability of a motor unit to produce force depends upon the cross sectional area of the constituent muscle fibres whereas the speed of contraction of a motor unit is dependent on the heavy-chain of the myosin protein in the muscle fibres.

When the motoneurone is excited to discharge action potentials, the muscle fibres of the motor unit are activated. The motor unit's action potential is the sum of action potentials propagated by all the muscle fibres that belong to that unit. The action potentials of the single fibres differ in amplitude and in frequency content, and they are temporally and spatially dispersed (van Eijden and Turkawski 2002).

After the synaptic transmission that occurs at the neuromuscular junction and which results in muscle contraction, action potentials are generated and which propagate along all the muscle fibres of a motor unit. All the muscle fibres of the motor unit contract approximately at the same time to result in a twitch contraction. Thus, the associated motor unit action potentials contribute to extracellular currents that sum to generate a field potential. This field can easily be recorded with electrodes placed on the skin over the muscle or via intramuscular electrodes (De Luca and Forrest 1973; Heckman and Enoka 2012).

The gradation of contraction of a muscle is controlled by two factors. First, the number of motor units that are recruited to participate in the act, and where more motor units are recruited, there will be a larger force generated from the muscle. Second, the frequency with which each motor unit fires action potentials determines the amount of force that each motor unit generates. Force summation occurs with increased firing of motor unit action potentials (van Eijden and Turkawski 2002). There is a threshold

value for the force required to activate the motoneurone where if force required in a movement or action is below this threshold value, then none of the muscle fibres in the unit will contract and if the force required in a movement or action is at the threshold value, then all the muscle fibres will contract (Hamilton et al. 2011).

1.4.8 Firing rates

As already mentioned, the magnitude of force produced by a muscle can be modulated by the central nervous system by two mechanisms:

(1) Varying the numbers of motor units that are recruited (recruitment gradation) and (2) Varying the discharge rates of action potentials of each motor unit (rate gradation) (van Eijden and Turkawski 2001).

The second way by which the force of a contraction can be modulated is, therefore, by changing the firing rates (FRs) of active motor neurones. By accurately decomposing the EMG signal into its constituent motor unit action potential trains (MUAPTs), it is possible to image the firing behaviour of motor units (Nawab et al. 2008).

In general, the firing rate of individual motor units is reported to increase with force increases. De Luca carried out studies (De Luca and Erim 1994) in which motor unit firing rates during ramp contractions (force trajectory that increased linearly from 0 to the target force at 10% MVC) were recorded and they observed that the firing-rate-force relationship can be characterized into three contiguous regions in the whole EMG activity.

1- Where the motor unit is newly recruited – the firing rate increases rapidly with force
2- Where a motor unit increases its firing rate more slowly as force increases and this coincides with the recruitment of new motor units
3- Where motor units increase their firing rates much faster than in the previous regions probably to compensate for the fact that motor unit recruitment is not available.

The decline in firing rate from recruitment to de-recruitment has been seen previously by recording single motor units during voluntary isometric contractions (Milner-Brown et al. 1973a) and is evidence of motoneuronal adaptation. And the de-recruitment order was orderly and the opposite of recruitment order (De Luca et al. 1982).

1.4.9 Recruitment threshold

A parameter which is commonly used to characterize motor unit recruitment as a function of motor unit size is the recruitment threshold (also force or activation threshold), in other words, the level of voluntary force for the first recruitment of a motor unit (Milner-Brown et al. 1973b).

In individual masticatory muscles, generally, the force produced cannot be measured directly, and the recruitment threshold is routinely defined by the bite force level at which the unit is activated (van Eijden and Turkawski 2001). For movements, the value of the threshold was defined as the magnitude (mm) of the jaw displacement at the point between the incisal edges of the lower central incisor teeth, namely, the mid-

incisor-point reference point of lower jaw movement in relation to the maxillae, along the most relevant axis for the analyzed movement phase. This definition has been used in a recent study of the medial pterygoid muscle (Chen et al. 2017) and in the lateral pterygoid muscle (Phanachet et al. 2001).

In fact, the recruitment threshold of a motor unit is not fixed but depends on several parameters, including contraction velocity (Budingen and Freund 1976), where studies show that with an increase in the speed of muscle contraction, the motor unit recruitment threshold force decreases (Budingen and Freund 1976; Duchateau and Enoka 2011; Freund et al. 1975; Tanji and Kato 1973), and muscle length (Miles et al. 1986). In fact, in their study, Pasquet et al. (Pasquet et al. 2005) actually found that motor units were activated at lower threshold at shorter muscle lengths. Another factor to be considered is the bite force direction (Hattori et al. 1991) and the duration of muscle contraction (Nordstrom and Miles 1991). Therefore, care is needed in using the bite force recruitment threshold as the only criterion for characterizing motor units.

1.4.10 Motoneurones driven by higher centres

The various functions related to the jaw muscles such as mastication and swallowing and the role they play in respiration and communication, are controlled by a complex sensorimotor system. This means that sensory imputs to the central nervous system provide feedback to guide and modulate those functions – See Figure 1-6 (Avivi-Arber and Sessle 2017).

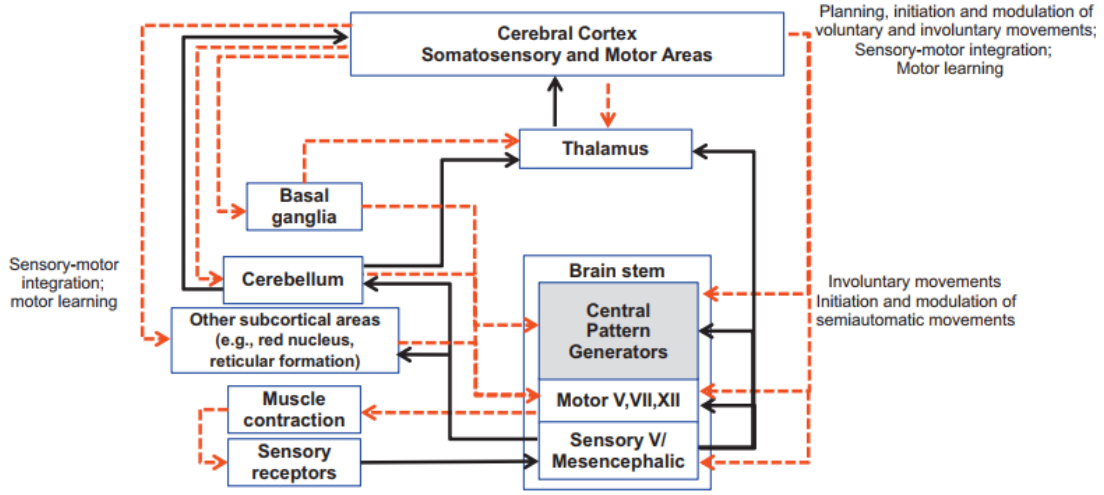


Figure 1-6: Principal inputs and outputs to and from face MI and face SI. There are considerable interconnections (excitatory and inhibitory), between the different cortical and subcortical regions, and commissural fibres are responsible for bilateral coordination. From: (Avivi-Arber et al. 2011).

Orofacial tissues have somatosensory receptors (including muscles spindles, Golgi tendon organs, other encapsulated endings and free nerve endings), which transduce external stimuli into action potentials that are conducted to the trigeminal brainstem sensory nuclear complex (VBSNC) for transmission to higher centres and further processing (Avivi-Arber and Sessle 2017).

Among those types of receptors, the free nerve endings usually are nociceptors and mostly respond to noxious (pain inducing) and thermal stimuli - morphologically, these receptors they lack specialized receptor cells or encapsulations. Because of this lack of specialization, the basis for their submodality specificity is unclear (Warren et al. 2013). The muscle spindles, in turn, have two types of primary afferent neurones (type Ia – that gives a sense of body position, namely proprioception, and a sense of body movement, namely kinaesthesia and type II – related with discharge when the muscle

is static). Lastly, the Golgi tendon is innervated by type Ib afferents and relates to signal contractile muscle tension (Avivi-Arber and Sessle 2017). Therefore, the muscle spindles and Golgi tendon organs respond to stretch and contractile tension, respectively but may also contribute with some nociceptive input into the CNS after some tissue injuries (Sessle 1999a; 2006).

Numerous primary afferents have neurone synapses on second-order neurones within the trigeminal brainstem sensory nuclear complex and other nuclei, such as the adjacent solitary tract nucleus (STN). The second-order neurones project to several other brainstem regions such as cranial nerve motor nuclei or adjacent interneuronal sites as well as to higher brain areas in the thalamus and the cerebral cortex for perceptual functions as well as for modulating orofacial movements.

The cerebral cortex, in particular the sensorimotor cortex (primary somatosensory cortex – S1 – and the primary motor cortex – M1) have important roles for sensorimotor functions. There are studies in the literature employing intracortical microstimulation (ICMS) or recordings of peripherally evoked responses and activity patterns related to movement of single neurones in the M1 and S1 of the cerebral cortex in monkeys (Huang et al. 1989; Martin et al. 1999), cats (Hiraba et al. 2007) and rats (Neafsey et al. 1986). In humans, stimulation, recording or imaging studies (Martin et al. 2004; Nordstrom 2007; Svensson et al. 2006) have also contributed for the understanding of the sensorimotor cortex function. This studies have shown the important role of the M1 in the planning, initiation and execution of limb movements and the contribution of the S1 to the control of the movements, and that both of them (M1 and S1) are fundamental

in the acquisition of new motor skills involving the limbs (Avivi-Arber et al. 2011; Sessle 2011).

In regards to the face area, studies in animals also show the importance of face M1 and face S1 in the generation and control of automatic, semiautomatic, and voluntary orofacial movements. M1 neurones project to the cranial nerve motor nuclei and are organised in somatotopic manner and as result of this, each microzone of the M1 represents a muscle (s) or movement (s). In fact, each microzone of the face M1 is intermingled with and frequently overlaps with other orofacial microzones as well as being next to microzones of the limbs and neck. Therefore, groupings of M1 microzones control movements involving the activation of more than one muscle, and intracortical electrical stimulation within different face M1 sites can also evoke different patterns of swallowing and/or mastication (Avivi-Arber et al. 2011).

A few factors suggest that some of the movement-related face M1 neuronal activity is a reflection of the sensory inputs generated by the orofacial movement and projected either directly or indirectly to the face M1. Therefore, a few factors reflect the important role of face M1 in the integration of sensorimotor information and the subsequent control of orofacial movements and semiautomatic movements (e.g., mastication and swallowing), previously mainly attributed to the CMA/swallow cortex and brainstem control mechanismsthe: the fact that the face M1 relays to sensory inputs both from directly (through the thalamic somatosensory or motor nuclei) or indirectly through the face S1 and the fact that blockade of somatosensory inputs to the face M1 by either peripheral nerve block, injury, S1 cold block or ablation can severely impair motor function (Avivi-Arber et al. 2011).

One of the unique features of mastication and swallowing are the central pattern generators (CPGs) that drive these movements. The CPGs involve a complex neuronal circuitry whereby the output of the involved neurones collectively provide the time-locked, patterned drive to the different motor neurone pools supplying the muscles that participate in chewing and swallowing.

The face M1 can also be related with pain. Imaging studies showed that painful electrical tooth stimulation (Jantsch et al. 2005) or hot stimulation of the skin overlying the masseter (de Leeuw et al. 2006) can elicit activation of the face M1. However, although hypertonic saline induced acute cutaneous pain may activate face S1 but not face M1, the same saline injections that induce acute pain in the muscle may activate face M1 as well as face S1 (Henderson et al. 2006; Kupers et al. 2004; Nash et al. 2010).

Neuroplasticity reflects the ability of the brain to adapt to peripheral alterations, recover from peripheral traumas or CNS injuries and to acquire motor skills and learning. This phenomenon can happen during the childhood, as an infant that develops new motor skills, as for example, in crawling or walking, or during adulthood as reflected by, for example, the ability to learn how to play a new sport or regain lost sesorimotor functions after peripheral or central injuries (Avivi-Arber et al. 2011).

However, it is important to elucidate that the neuroplasticity does not necessarily represent a beneficial adpatative modification. For instance, maladaptation that may

lead to chronic dysfunctions can happen and one example of such maladaptation is in TMD (Avivi-Arber et al. 2011).

There is some evidence that the primary motor cortex is involved in a redistribution of this motor activity during noxious stimulation (Murray and Peck 2007). It is well-known that neuroplastic changes occur in sensorimotor cortical areas following peripheral manipulations of sensory inputs into and motor outputs from the brain (Sanes and Donoghue 2000; Sessle 2006; Toldi 2008). Spinal pain-imaging (Casey 1999; Graven-Nielsen et al. 1997d) and electrophysiological (Farina et al. 2001; Le Pera et al. 2001; Valeriani et al. 2001; Valeriani et al. 1999) studies show that nociceptive activity inhibits limb primary motor cortical excitability. There is recent evidence that the face area of the primary motor cortex (that drives voluntary orofacial movements) is markedly affected by noxious stimulation. For example, tongue mucosal pain interferes with motor cortex neuroplasticity associated with novel tongue-task motor training (Boudreau et al. 2007). Noxious lingual stimulation results in prolonged (>4 hours) neuroplastic changes manifested as profound decreases (300% increases in thresholds) in the excitability of the somatotopically relevant region of the face primary motor cortex in the rat (Adachi et al. 2008) and analogous findings have been reported in limb motor cortex studies (Farina et al. 2001; Le Pera et al. 2001). We have also shown, through functional magnetic resonance imaging (fMRI) (Nash et al. 2010) that noxious stimulation of human facial skin or masseter muscle evoke long-duration (>6 minutes) decreases in face motor cortex activity.

Face M1 neuroplasticity has also been reported in chronic orofacial pain conditions such as trigeminal neuralgia (DaSilva et al. 2008) and patients reporting parafunctional

oral motor habits such as clenching and grinding have decreased fMRI activation within M1 during clenching (Byrd et al. 2009). Therefore, as it was already mentioned that motor training may produce M1 neuroplasticity, applying training to reorganize the sensorimotor cortex can possibly be an effective approach to improve motor control in chronic orofacial pain patients (Sessle 2011).

The pathophysiology and mechanism of pain are explained further on section 1.7

1.5 Concepts of the relation between pain and muscle activity

For many years, clinical diagnosis and management of musculoskeletal pain conditions have been influenced by the notion of a simple, reflex-like association between pain and muscle activity (Murray and Lavigne 2014; Svensson and Graven-Nielsen 2001).

1.5.1 The Vicious Cycle Theory (VCT)

The Vicious Cycle Theory (VCT), often cited in clinical practice, proposes a positive interrelationship between pain and so-called muscle “hyperactivity” (Figure 1-7). Therefore, an initiating factor such as an abnormality in structure, posture, movement, or stress results in pain that causes what is called “muscle hyperactivity,” which, in turn, leads to spasm or fatigue and further pain and dysfunction, and perpetuating the cycle (Travell et al. 1942).

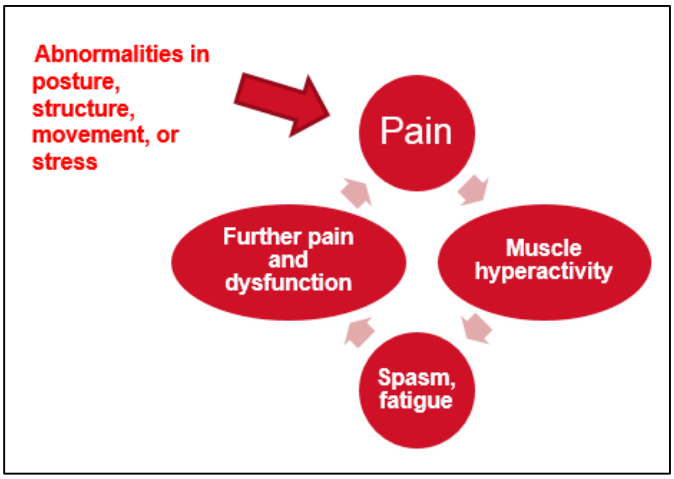


Figure 1-7: Schematic figure of how the Vicious Cycle Theory works to perpetuate pain.

Management strategies based on this theory attempt to break this cycle by, e.g., irreversible and often expensive changes to the anatomy (e.g. surgery, tooth adjustments) (Stohler 1999).

However, the validity of the idea proposed by the theory is controversial and not clinically proven. Contrary to the theory's fundamental key assumption that painful muscle is hyperactive, the literature has shown a considerable amount of evidence from clinical and experimental studies in the spinal and trigeminal systems, revealing only small differences (either increases or decreases or no change) in resting EMG activity between painful and non-painful muscle (Ahern et al. 1988; Bodere et al. 2005; Carlson et al. 1993; Collins et al. 1982; Graven-Nielsen and Arendt-Nielsen 2008; Graven-Nielsen et al. 1997d; Madeleine and Arendt-Nielsen 2005; Majewski and Gale 1984; Peck et al. 2008; Sae-Lee et al. 2008b; Sherman 1985; Simons and Mense 1998; Stohler 1999; Stohler et al. 1996; Svensson et al. 1998; Thomas Graven-Nielsen et al. 2000; van Dieen et al. 2003; Xu et al. 2010). And the clinical significance of any of these small changes is questionable.

1.5.2 The Pain Adaptation Model (PAM)

The Pain Adaptation Model (PAM) (Figure 1-8) (Lund 2008; Lund et al. 1991) on the other hand, is another widely cited model that proposes that pain results in slower and smaller movements so as to minimise further injury and therefore aids healing (Campbell et al. 2010; Lund 2008; Stohler 1999). According to this model, pain would change the excitability of motoneurones and therefore cause an alteration in muscle activity, namely an inhibition of agonist muscle activity and facilitation of antagonist muscle activity (Lund et al. 1991). 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 (Lund et al. 1991).

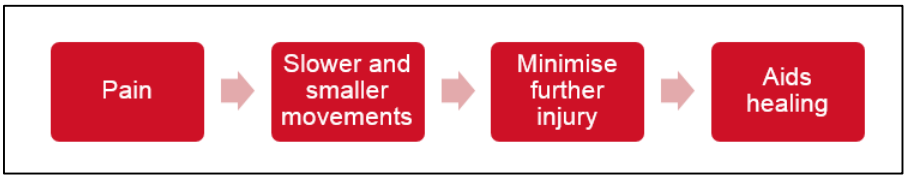


Figure 1-8: Schematic figure of how the adaptation proposed by the Pain Adaptation Model would help to aid in healing.

Management strategies based on this model invoke pharmacological and behavioral strategies to reduce pain and minimize movement to allow the jaw motor system to heal and recover (Fricton and Schiffman 2008). This model, however, does not explain the possible origin of the pain. In addition, studies in both the spinal and trigeminal systems provide limited evidence in support of this model (Hodges et al. 2015; Hodges and Tucker 2010; Murray and Peck 2007). For example, an earlier study of experimental masseter muscle pain induced by hypertonic saline injections was unsuccessful in confirming the uniform inhibition of agonist muscle activity and facilitation of antagonist muscle activity as proposed by the model (Sae-Lee et al.

2008a). Another study showed reduced activity in both the agonistic and antagonistic muscles during muscle pain, without significantly impairing the movement amplitude or acceleration (Ervilha et al. 2004). Recordings from the knee extensor and flexor muscles in a force-control and a position-control task during pain have demonstrated increased agonist muscle activity while the antagonist muscle EMG activity only increased in the force-control task (Poortvliet et al. 2015). Antagonist muscle activity did not increase in other studies (Birch et al. 2000; Falla et al. 2007).

In fact, accumulating evidence indicate that neither of these earlier theories provides an adequate explanation of the association between pain and muscle activity (Lund 2008; Murray and Lavigne 2014; Murray and Peck 2007; Murray et al. 2014; Stohler 1999; Svensson and Graven-Nielsen 2001). As indicated below, other factors may be playing a role in influencing the relation between pain and motor activity, e.g. psychological factors.

1.5.3 Reorganization of single motor unit activity within muscles during pain

A major problem with the proposals of these earlier theories is that they propose uniform increases or decreases of activity within a painful muscle. However, it is difficult to reconcile such uniform changes in muscle activity with the clinical observations of localised tenderness within painful jaw muscles in many TMD patients.

Recent experimental muscle pain studies in limb muscles, however, have provided good evidence that complex, non-uniform changes in activity can occur within

individual muscles. For example, a slowing and/or de-recruitment of one population of motor units, together with a recruitment of a new population of units, has been demonstrated within painful limb muscles (Hodges and Tucker 2011; Tucker et al. 2009). It is also important to mention that muscle function may also affect the pain due to an impact on the volume injected / infused. For example, Kumar et al., 2015 in their study, reported that the possibility of diffusion of the injected monosodium glutamate into the muscle during muscle contraction for the performance of the behavioral task, may lead to an enhanced washout of the solution and thereby reduce the effect of the painful stimulus (Kumar et al. 2015).

In a study of limb muscle behaviour in pain (Tucker et al. 2009), a total of 52 SMUs were discriminated in the quadriceps and 34 SMUs were discriminated in the flexor pollicis longus (FPL) muscle during low-force contractions in both pain into the infrapatellar fat pad and no-pain trials. Of these, 20 quadriceps and 9 FPL units were identified during both pain and no-pain trials (see Figure 1-9). All remaining units discharged only with or without pain, but not in both conditions. One-third of the additional units were recruited during pain – See figure 1-9 and 1-10.

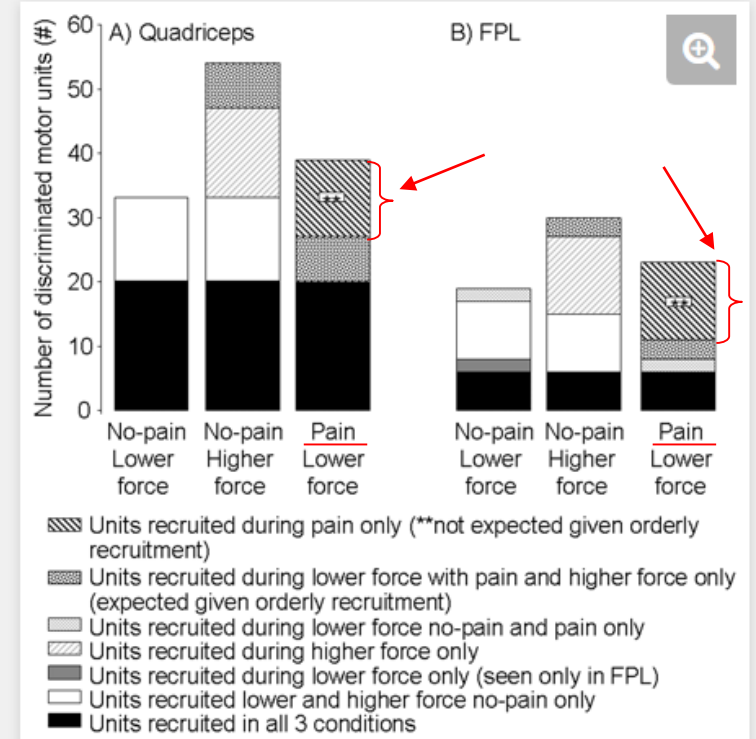


Figure 1-9: Number of discriminated motor units in quadriceps (A) and flexor pollicis longus (B) during three contraction conditions (no-pain lower force, no-pain higher force, pain lower force). Observe in black the number of units that were recruited for all the conditions and an arrow was placed to point out the units that were recruited only for the pain condition. From: (Tucker et al. 2009).

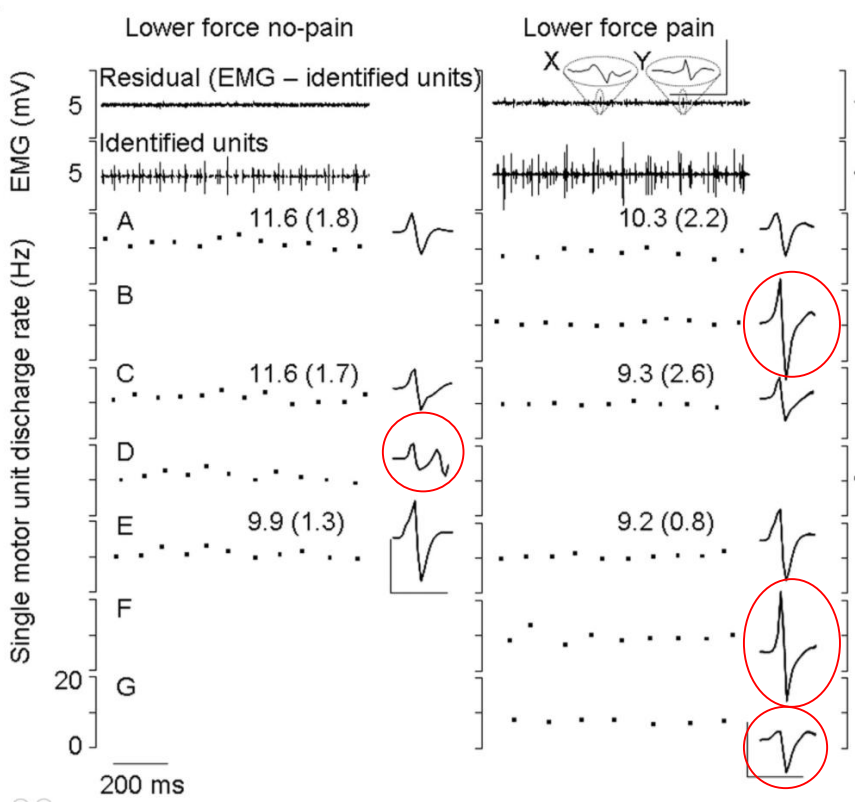


Figure 1-10: Recordings of motor unit activity from a fine-wire electrode inserted into the FPL of one subject. SMUs are shown with their respective discharge rates [mean (SD)]. A red circle points out the units that were recruited in the lower force pain condition (units B, F, G) and that were de-recruited during the lower force pain condition (Unit D). From: (Tucker et al. 2009).

There is more evidence in the literature that muscles other than the painful one can change their activity to maintain the motor output (Ervilha et al. 2005; Muceli et al. 2014). For example, Muceli, Falla and Farina, in 2014 published a study where they injected hypertonic saline into the right anterior deltoid muscle and which resulted in a decrease in its activity with pain. But the saline injection also resulted in alterations in the activity (increase or decrease) of muscles other than the one injected with saline and the effects were subject specific (Muceli et al. 2014). Other studies have also identified changes in EMG activity (greater activity or decrease in activity) in different non-painful leg muscles with noxious stimulation of the spinous process or gastrocnemius medialis (van den Hoorn et al. 2015).

Changes in non-painful jaw muscle activity have also been observed with algesic chemical injections into isolated jaw muscles (Costa et al. 2017; Kumar et al. 2015a; Sae-Lee et al. 2008a; Svensson et al. 1996a). Also, Shimada, Hara and Svensson in 2013, studying the masseter and temporalis muscles, injected hypertonic solution into the right masseter muscle and observed that EMG activity decreased in the painful muscle and increased in other muscles at different levels of clenching (Shimada et al. 2013). Sae lee et al in 2008 studying the EMG activity of bilateral masseter, and right posterior temporalis, anterior digastric, and inferior head of lateral pterygoid muscles also found that EMG activity were significantly effected by hypertonic saline-induced pain in the masster depending on the task in which the muscle participated irrespective of whether the muscle was an agonist or an antagonist in the tasks (Sae-Lee et al. 2008a).

While studies of the effects of noxious stimulation of the jaw muscles have focussed on surface EMG recordings from the jaw muscles and which provide evidence for overall changes in jaw muscle activity in pain, there is very little information as to whether the reorganization of motor unit activity which has been demonstrated in the spinal motor system (for review – See Hodges et al., 2015) also operates in the trigeminal motor system.

We have recently provided evidence that the presence of experimental pain in the masseter muscle (a jaw closing muscle) results in a reorganization of activity, that is, increases and decreases of motor unit activity occurs within the same painful muscle (Minami et al. 2013). In this study, Minami at al in 2013 discriminated 36 SMUs, and

from these, 21 were present in both conditions (pain and no-pain), while 5 were present only in the no-pain condition and 10 were present in the pain condition only (see Figure 1-11) (Minami et al. 2013).

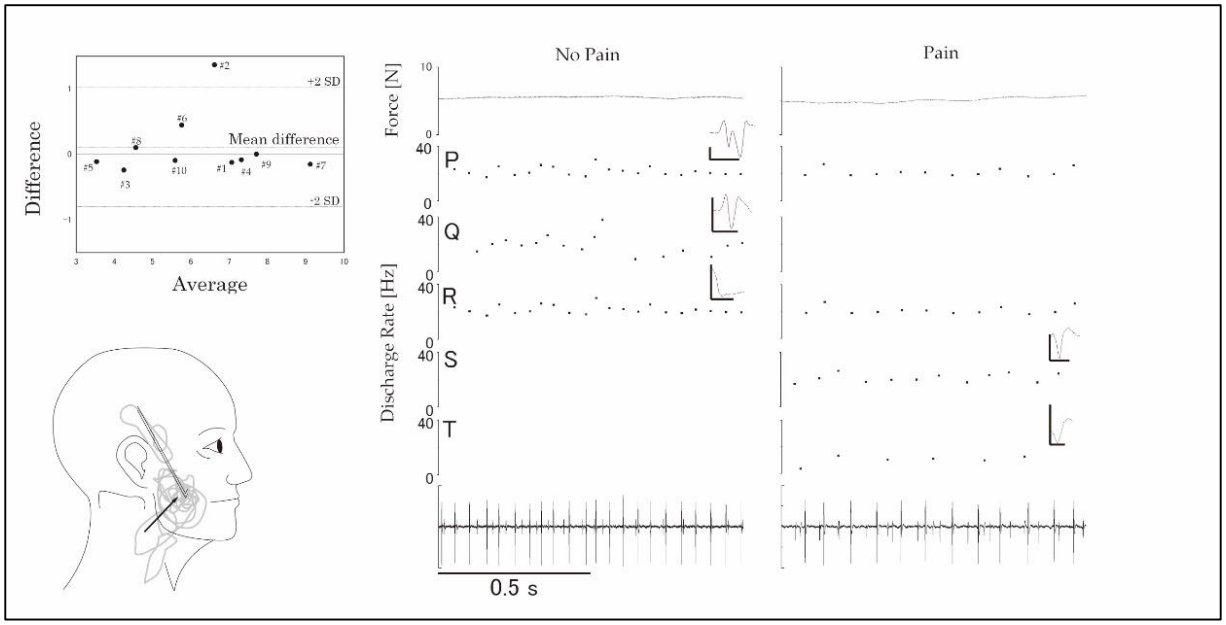


Figure 1-11: Top left: Bland-Altman plot showing mean difference in force levels between pain and no-pain trials. Lower left: Injection site (arrow), needle electrodes for recording single motor unit activity, pain maps during hypertonic saline infusions; Right panel: single motor units P, Q, R were present under no pain trials, but during pain trials, single motor units P, R, S and T were present. Therefore, 2 SMUs were recruited in pain and 1 SMU was de-recruited (reproduced from (Minami et al. 2013).

Also, Malik (Malik 2016) recorded SMU activity at 2 sites within the right masseter muscle (a superior/anterior site - RMS/RMA, and an inferior/posterior site - RMI/RMP) during isometric biting tasks under 3 sessions: baseline (no infusion), 5% hypertonic saline infusion (pain), and isotonic saline infusion (control). At the RMI/RMP site and for the comparison of hypertonic saline infusion with isotonic saline infusion, of the 58 instances where all the SMUs were tested for recruitment or de-recruitment in a task, there were 7 instances of recruitment of new SMUs and 7 occurrences of de-

recruitment of SMUs. For the 68 instances where SMUs were tested at the RMS/RMA site, there was 1 recruitment and 2 de-recruitments.

These data from these recent studies (Malik 2016; Minami et al. 2013) represent the first demonstration of reorganization of SMU activity within the masseter muscle in the jaw muscle system. These single motor unit studies in the jaw motor system have, however been restricted to studies of motor unit activity within the muscle that is receiving the algesic chemical injection. However, as summarized above, studies in the limb motor system have demonstrated a reorganization of SMU activity can occur even in non-painful muscles (Tucker et al. 2009). To date, there have been no studies as to whether the reorganization of single motor unit activity that has been demonstrated within the masseter muscle during noxious stimulation of the masseter (Malik 2016; Minami et al. 2013) leads to a reorganization of single motor unit activity within other jaw muscles, e.g. the temporalis muscle, another major jaw closing muscle. This is important to study because patients with TMD frequently have pain and tenderness not only in the masseter muscle but also other jaw muscles, such as the temporalis and medial pterygoid muscles. Studying the activity patterns of SMUs in non-painful muscles in an experimental muscle pain paradigm might provide possible insights into the spread of pain in TMD if some of these possible changes in muscle activity might predispose to further pain and tenderness in these non-painful muscles. Such possible pain effects associated with changes in motor activity have been proposed in the spinal motor system (Hodges et al. 2003) and also suggested in the jaw motor system (Murray and Peck 2007).

Nonetheless, these new data (Malik, 2016; Minami et al., 2013) point to the need to re-assess management strategies for patients with jaw muscle pain conditions which are

based on the earlier theories (i.e. Vicious Cycle Theory, Pain Adaptation Model) (Lund 2008; Murray and Lavigne 2014) and which propose generalized reductions or generalized increases in EMG activity within muscles and where pain is present either within the muscle or in adjacent structures. Rather than supporting these earlier models, the findings tend to support other models that propose that in the presence of pain muscle activity undergoes a reorganization (Hodges and Tucker 2011; Murray and Lavigne 2014; Murray and Peck 2007).

1.5.4 Possible role of psychological factors in the relation between pain and muscle activity

Another problem with the earlier theories (namely, the Vicious Cycle, the Pain Adaptation Model) is that they take no account of the possible role of psychological factors in the relation between pain and muscle activity. In recent years, there is increasing acceptance that psychological factors play an essential role in this interaction (Hall et al. 2011; Murray and Peck 2007; Ohrbach et al. 2011) as well as playing an important role in the onset and progression of chronic pain conditions (Innes 2005; Kuch 2001). In many case-control studies, investigators have compared pain-free controls and patients with chronic pain conditions and a common finding from all studies shows that patients with chronic pain conditions show elevations on measures of psychosocial distress, environmental stress, catastrophizing, and somatic awareness (Fillingim et al. 2011; Gatchel et al. 2007; Hirsch and Turp 2010; Keefe et al. 2004; Nevalainen et al. 2016; Tournavitis et al. 2017).

The psychological factors have been implicated particularly in the transition from acute to chronic pain and disability in chronic low back pain (Hall et al. 2011). Patients with

TMD also report higher levels of affective distress (Reissmann et al. 2008), somatic awareness (Lei et al. 2015), psychosocial stress (Ajilchi and Nejati 2017) and pain catastrophizing (Nilges and Essau 2015).

Pain catastrophizing is one psychological variable that has received considerable attention in recent years (Leeuw et al. 2007; Nicholas et al. 2011; Sullivan et al. 2001; Sullivan MJL 1995). Pain catastrophizing is the cognitive element of the fear network and refers to the process whereby pain is interpreted as being extremely threatening. High catastrophizing individuals have a tendency to focus excessively on a pain sensation (rumination), to exaggerate its threat (magnification), and to experience a sense of helplessness (Leeuw et al. 2007; Sullivan et al. 2001).

One of the theoretical models incorporating catastrophizing is the Fear Avoidance Model of musculoskeletal pain (Figure 1-12) (Leeuw et al. 2007; Sullivan et al. 2001; Wideman et al. 2013). This model proposes that high catastrophizing individuals react to an acute pain episode by becoming fearful about movements and therefore develop safety seeking or avoidance behaviours (i.e. new movements and muscle activity patterns) that can be adaptive in the acute stage (by reducing pain briefly) but paradoxically can lead to disuse, disability, depression and further pain in the long term (Figure 1-12, left side of figure) (Leeuw et al. 2007; Sullivan et al. 2001).

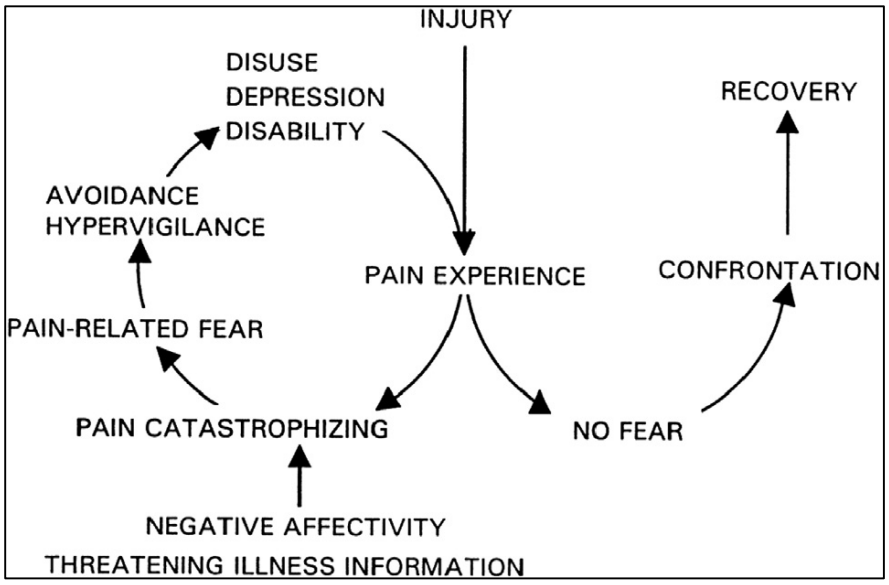


Figure 1-12: Fear Avoidance Model. Reproduced from: (Wideman et al. 2013).

Low catastrophizing individuals, on the other hand, according to the Model (Figure 1-12, right side), perceive acute pain as non-threatening, do not develop an unrealistic fear of movement and they confront the pain and this promotes functional recovery.

Despite the attention given to catastrophizing in explaining trunk and limb musculoskeletal pain problems, much less attention has been given to catastrophizing and fear of movement in the jaw musculoskeletal system, although there is emerging evidence for pain catastrophizing in pain persistence, disability and treatment failure in TMD (Nicholas et al. 2008; Velly et al. 2011; Visscher et al. 2010).

Therefore, this Fear Avoidance Model may be useful in explaining the transition from acute to chronic TMD. However, we do not know how well the Model provides a theoretical basis for understanding the progression of TMD. For example, we do not

know whether pain catastrophizing influences the effects of orofacial pain on jaw movements and jaw muscle activity patterns (i.e. avoidance behaviours), and whether these activity patterns can actually contribute to the progression of the TMD pain condition, a key proposal of the Fear Avoidance Model.

There is some evidence in the trigeminal system, however, that pain catastrophizing plays a role in the initiation and/or progression of orofacial pain or plays a role in the interaction between pain and motor activity. This evidence includes clinical studies (Turner et al. 2002) and experimental pain studies (Akhter et al. 2014; Henderson et al. 2016; Kristiansen et al. 2014; Trost et al. 2015). Akhter et al., 2014 investigated differences between higher and lower pain catastrophizers in the effects of hypertonic saline-evoked jaw muscle pain and found an increased report of pain intensity, pain areas and McGill Pain Questionnaire pain rating indices consistent with enhanced central sensitization processes in higher catastrophizing individuals.

Catastrophizing, fear-avoidance and depression have also been shown to correlate significantly with subjective or objective measures of motor performance. (Alschuler et al. 2008; Castaneda et al. 2008; Leeuw et al. 2007; Thomas et al. 2008; Turner et al. 2001). For example, individuals with high pain-related fear have smaller peak velocities and accelerations of the lumbar spine and hip joints in reaching trials (Thomas et al. 2008). There is recent evidence that catastrophizing and depression contribute to the progression of chronic TMD pain and disability, (Velly et al. 2011) and that high intensity muscle pain is a predictor for the development of chronic TMD (Epker et al. 1999). We have also recently demonstrated significant correlations between psychological variables (e.g. catastrophizing, depression, and orofacial pain intensity and quality) and jaw muscle and jaw movement features and brain activity features

(Akhter et al. 2014; Brandini et al. 2011; Henderson et al. 2016; Murray and Peck 2007).

There is little information however whether some of these psychological variables, such as pain catastrophizing, plays a role in influencing the relationship between pain and motor unit activity at the single motor unit level in terms of, for example, the reorganization of single motor unit activity that appears to be occurring within painful jaw muscles or within non-painful muscles during painful jaw muscle stimulation.

1.6 The Integrated Pain Adaptation Model (IPAM)

Given the limitations of the earlier models of pain-motor interaction, it has been proposed that there is a more complex explanation for the effect of pain on motor activity. In fact, according to The Integrated Pain Adaptation Model (IPAM), the effect of pain on motor activity depends on the interaction of the individual's biopsychosocial variables with the individual's pain experience (i.e., the multidimensional nature of pain) and the anatomical and functional complexity of the individual's sensory-motor system (Figure 1-13) (Murray and Peck 2007; Sae-Lee et al. 2008b).

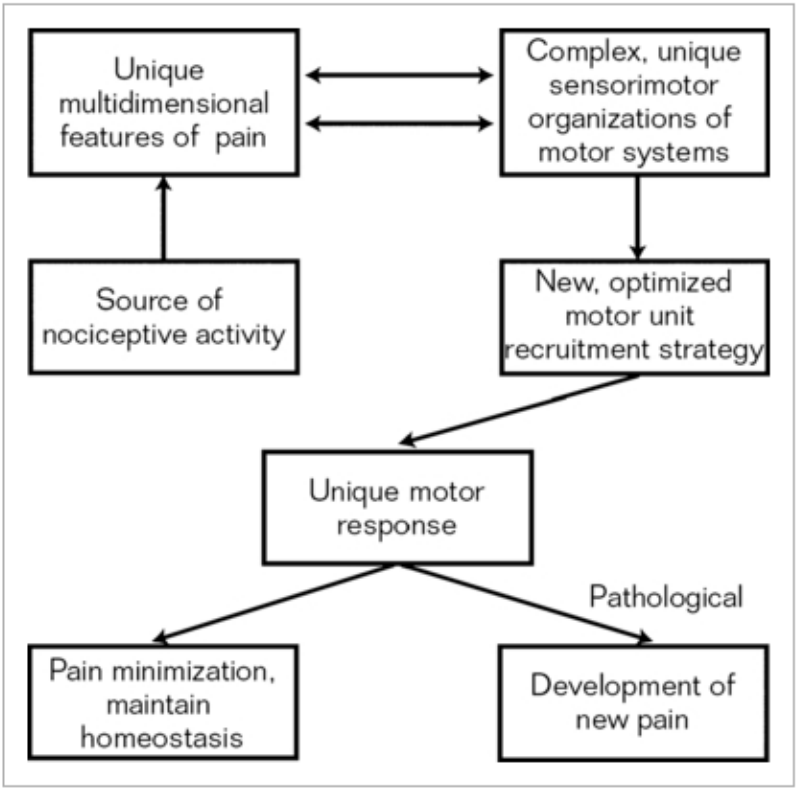


Figure 1-13: Diagram outlining essential components of the Integrated Pain Adaptation Model (IPAM). Extracted from (Sae-Lee et al. 2008b).

For instance, the Integrated Pain Adaptation Model suggests that the interaction of those variables that are part of the complexity of the individual's pain experience (e.g., acute/chronic, muscle/joint, beliefs based on past experience, catastrophizing, emotional contributions, motivation, social context, genetic factors) will singularly affect motor activity in a unique way. In other words, if the individual's experience of pain varies, the individual's motor response to pain will also be varied.

Consequently, the way that motor units are recruited may be individually reorganized in order to preserve homeostasis or minimize an individual's pain experience, yet maintaining the capacity of the individual to perform motor activities, e.g. a masticatory

action. Thus, when jaw muscles are painful, the motor unit recruitment strategy adopted to achieve mastication could vary among individuals depending on the interaction with these other variables. Further research is required to elucidate those pain-related variables that have important influences on jaw muscle function (Brandini et al. 2011; Sae-Lee et al. 2008b).

Therefore, according to this model, it is not suitable to manage chronic pain patients in exactly the same way, regardless the fact that they might have the same physical diagnosis (Sae-Lee et al. 2008b). As a matter of fact, individualizing management based on the multidimensional pain experience with a focus on psychosocial status rather than the physical pain experience by itself has demonstrated successful outcomes (Dworkin et al. 2002).

1.7 Pathophysiology and mechanisms of pain

Generally speaking, pain varies in character, location, duration and intensity. In order to have a better understanding of pain, it is important to recognize some definitions. Therefore, a noxious stimulus is considered to be a stimulus capable of inflicting damage or threatening to damage tissues (Warren et al. 2013).

As already mentioned, receptors for touch, pressure, innocuous thermal stimuli, and nociceptive stimuli are distributed in the skin and in deep tissues. Sensory neurones that innervate the skin, viscera, deep muscle, and joints are called primary afferents. These neurones have their soma in the dorsal root or trigeminal ganglia and extend

axons into their peripheral targets and centrally into the spinal cord or brainstem. When peripheral body tissues are activated either by non-noxious thermal or mechanical stimuli or noxious stimuli, the somatosensations are conducted in the form of action potentials to the CNS via primary afferent nerve fibres which travel within peripheral nerves (L. Ingram 2017).

The detection of the nociceptive environment and inflammatory mediators is accomplished through many specialized receptor proteins that are differentially expressed in primary afferents. Nociceptive activity generally occurs with tissue damage and is a result of the release of neurotransmitters, cytokines, and growth factors from adjacent blood vessels, damaged cells and others, which activate and sensitize nociceptive nerve terminals (Haas and Lennon 1995) – See figure 1-14. The transduction, therefore, starts when these proinflammatory mediators interact with the ion channels and receptors that are expressed on the peripheral nerve membrane of the nociceptor (Hunt and Mantyh 2001).

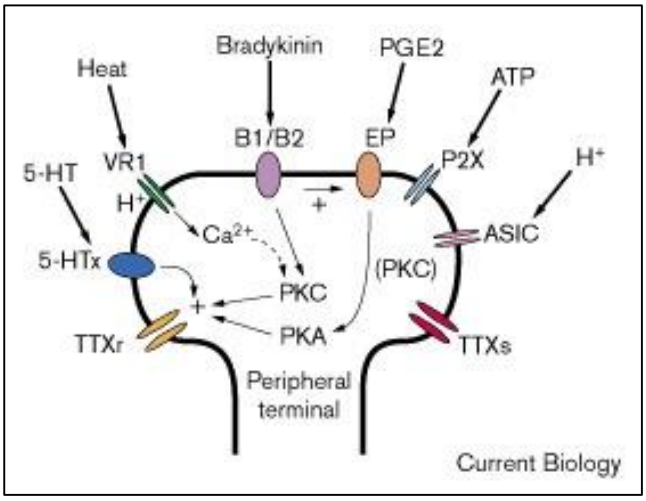


Figure 1-14: The peripheral terminal of a nociceptor, showing some of the various ligand-gated ion channels and G protein-coupled receptors, and the inflammatory mediators that bind directly to them. From: (Basbaum and Woolf 1999).

Afferent nerve fibres convey information from somatosensory receptors in the head and body and ascend from their peripheral location through the trigeminal ganglion and dorsal root ganglia and on through relay centers to higher centres where the information is processed and the patient may perceive the neural information as the perception of heat or cold, touch or pressure or pain. The afferent fibres are classified into three main groups according to their conduction velocities, function and degree of myelination (Basbaum and Jessell 2000) –See Table 1-1 and figure 1-15.

- A beta (Aβ) fibres are myelinated and have fast conduction velocities (35-120 m/sec). These fibres transmit mechanosensory information and although this fibre type does not transmit nociceptive information, recent studies have demonstrated that they may have a role in chronic pain states.
- Nerve fibres that transmit nociceptive and thermal information are known as A delta and C fibres. A delta (Aδ) fibres are lightly myelinated and have relatively fast conduction velocities (5-30 m/sec) when compared to C fibres and evoke sharp localized pain responses.
- C fibres are unmyelinated and have slower conduction velocities (0.5-2 m/sec) and are responsible for the dull, diffuse, burning pain (L. Ingram 2017) (James et al. 2017).

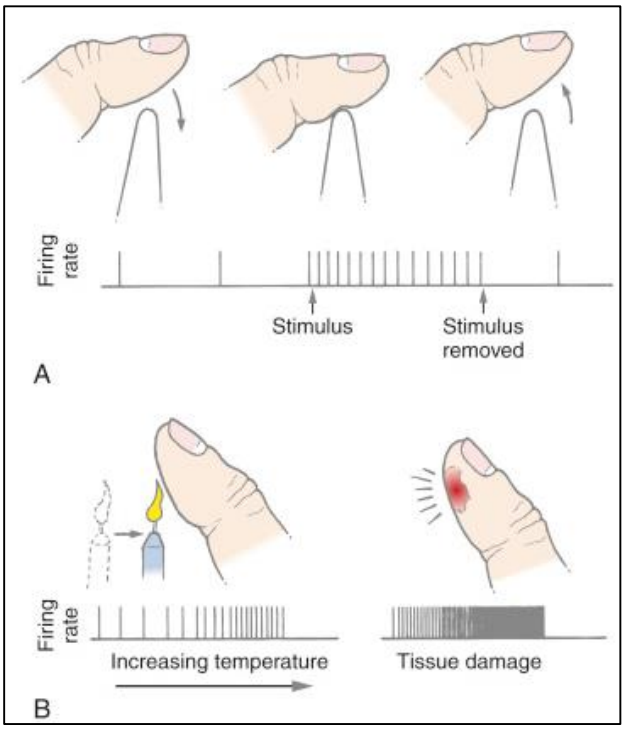


Figure 1-15: Fibres conveying information from high-threshold mechanoreceptors (**A**) respond to the application of a punctate stimulus. Thermoreceptors show a response that increases while temperature increases (**B** , *left*), while burns caused by prolonged thermal stimulation, for example, evoke high-frequency response in thermonociceptors (**B** , *right*). From: (Warren et al. 2013).

Type of nerve fibre	**Information carried**	**Myelin sheath**	**Diameter (micrometers)**	**Conduction speed (m/s)**
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

Table 1-1: classifications of peripheral afferent nerve fibres (From Dubner et al. 1978).

These noxious stimuli that can evoke a sensation of pain in humans include mechanical stimuli (e.g. heavy pressure), algesic chemicals, and inflammatory agents (Sessle and Hu 1991). Ischemia also could act as a stimulus if it is prolonged enough and associated with muscle contractions (Sessle 1999a).

Nociceptors, therefore, respond to noxious stimuli applied to the peripheral tissues, and are associated with A-δ and C fibres, which in turn, conduct information about these nociceptive events to the central nervous system for processing - for review see (Sessle 2009). It is important to mention that many C fibres have been found to act not only in respond to noxious stimuli, but also to multiple stimulus modalities such as non-noxious cool or warm stimuli (Lawson et al. 2008; Van Hees and Gybels 1981).

In fact, multiple pathways in the CNS are involved in the processing of noxious stimuli for the perception of pain. Studies of human brain imaging have revealed consistent cortical and subcortical networks that are activated by noxious stimuli, including sensory, limbic and associative regions. The brain areas activated by noxious stimuli in human brain imaging studies are typically the primary somatosensory cortex (S1), secondary somatosensory cortex (S2), anterior cingulate cortex (ACC), insula, prefrontal cortex (PFC), thalamus and cerebellum – See figure 1-16. (Apkarian et al. 2005; Bushnell et al. 2013).

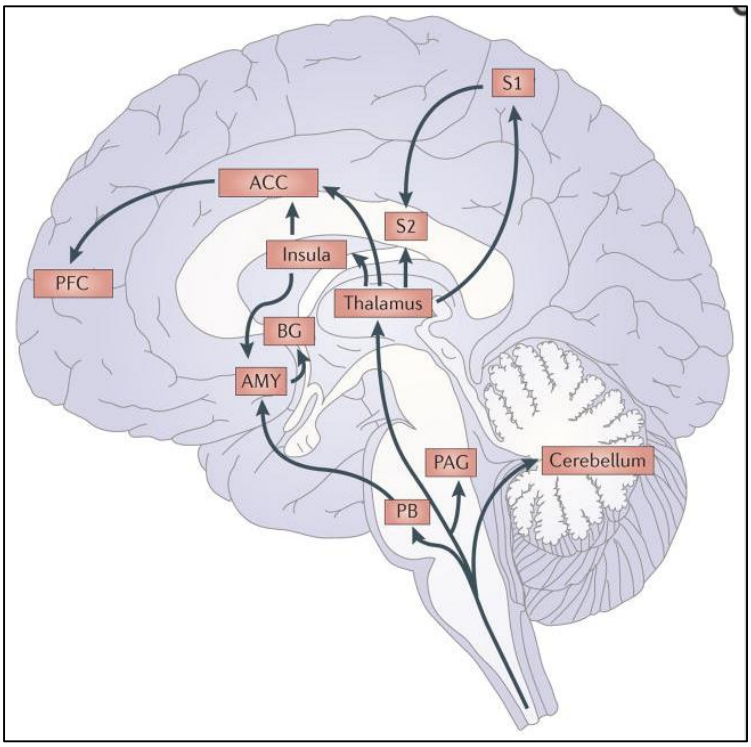


Figure 1-16: "Afferent pain pathways and brain regions. Afferent nociceptive information enters the brain from the spinal cord. Afferent spinal pathways include the spinothalamic, spinoparabrachio–amygdaloid and spinoreticulo–thalamic pathways. Nociceptive information from the thalamus is projected to the insula, anterior cingulate cortex (ACC), primary somatosensory cortex (S1) and secondary somatosensory cortex (S2), whereas information from the amygdala (AMY) is projected to the basal ganglia (BG). See the main text for references. PAG: periaqueductal grey; PB: parabrachial nucleus; PFC: prefrontal cortex." From: (Apkarian et al. 2005).

The somatosensory cortices (S1 and S2) are thought to encode information about sensory features, such as the pain location and duration (Chudler et al. 1990; Kenshalo et al. 1988; Kenshalo and Isensee 1983). The ACC and insula, on the other hand, have been considered components of the limbic (emotional) part of the brain (Mac 1949), and consequently are more important for encoding the emotional and motivational aspects of pain.

Within the orofacial area, the majority of nociceptive information in the head and neck is transmitted via branches of the fifth cranial nerve—the trigeminal nerve, which is considered the largest of the cranial nerves (Dubner et al. 1978; Fields 1987; Sessle 2005; Sessle 2009) – See figure 1-17. Other nerves including facial, glossopharyngeal, vagus, hypoglossal and the upper cervical spinal nerves (C1-C3) are also involved in conveying input to higher centres (Aghabeigi 2002).

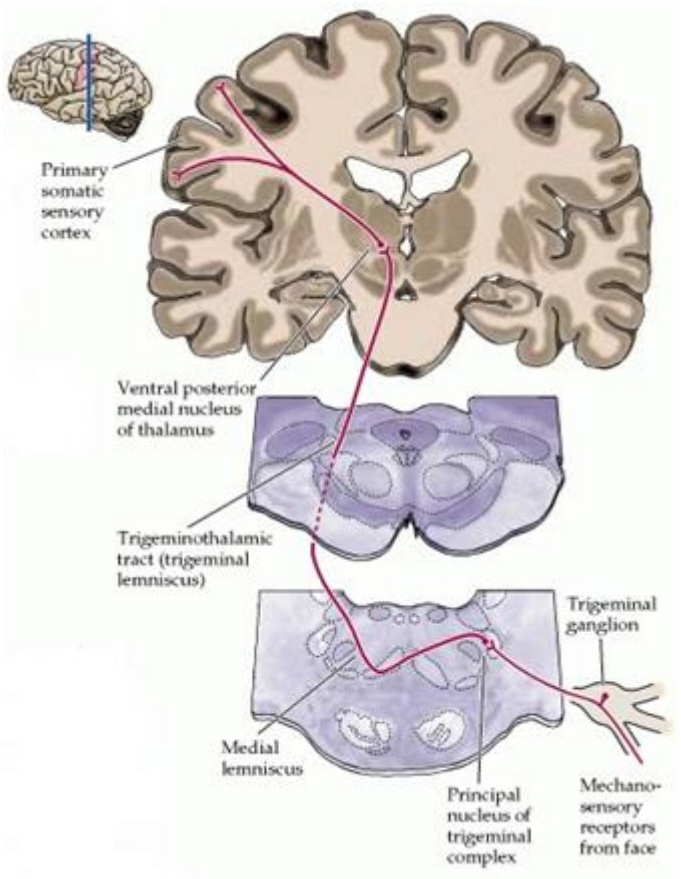


Figure 1-17: Schematic representation of the main mechanosensory pathways. The trigeminal portion of the mechanosensory system carries information from the face. From: (Purves D 2001).

Subsequently after the initial nerve signals have been produced, the information travels along the trigeminal primary afferent nerve fibres which project to the VBSNC where they can go up or down in the trigeminal spinal tract. The afferent nerve fibres give rise

to collaterals that end up in one or more subdivisions of the VBSNC and may activate second order neurones within or next to the trigeminal brainstem complex. This complex also can receive afferent inputs from other cranial nerves and from upper cervical nerves that additionally transmit afferent inputs from the upper cervical dorsal horn (Darian-Smith 1966; Dubner et al. 1978; Sessle 2000; 2006; Sessle 2009).

The VBSNC can be subdivided into the principal or main sensory nucleus and the spinal tract nucleus, which consist in three subnuclei: oralis, interpolaris, and caudalis (Sessle 2000) – See figure 1-18.

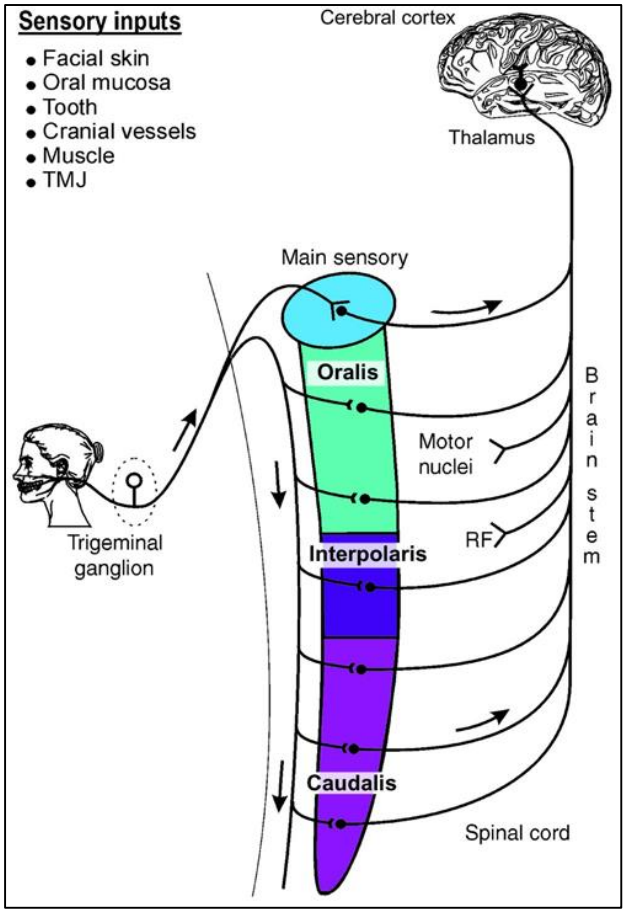


Figure 1-18: The spinal tract nucleus pathways whereby trigeminal somatosensory information enters the brain can be subdivided from rostral to caudal into 3 categories: subnucleus oralis, interpolaris and caudalis. The nociceptive information from the orofacial area is transmitted via the branches of the trigeminal nerve (cranial nerve V), which cell bodies are located in the trigeminal ganglion. Information

is further processed as it courses through second-order neurones in the spinal trigeminal nuclear complex within the brainstem, and then the nociceptive information ascends to the thalamus and cerebral cortex (Sessle 2000).

Research data show that most of the nociceptive information related to the trigeminal system is received by the subnucleus caudalis and it has a close structural and functional relationship with the spinal region (Sessle 1999a). Also, anatomical studies have indicated that the subnucleus caudalis extends into the cervical spinal cord and merges with the spinal cord dorsal horn which is an integral component for spinal nociceptive transmission. Both regions comprise a laminated structure with similar second-order neurone types that project to the thalamus. Thus, the term medullary dorsal horn is designated for the subnucleus caudalis considering these similarities (Hannam and Sessle 1994).

The second-order nociceptive neurones are located in the superficial (*e.g.,* lamina I) and deep (*e.g.,* lamina V) layers of the medullary dorsal horn and could be divided, according to their cutaneous mechanoreceptive properties, into two types: wide dynamic range (WDR) or nociceptive specific (NS) neurones (Hannam and Sessle 1994). These neurones can be activated by the stimulation of a range of afferents from articular and muscular tissues along with cutaneous and mucosal tissues (Sessle 1999a).

The WDR neurones can be activated by both noxious and innocuous stimuli while the NS neurones are activated entirely by noxious thermal and/or mechanical stimuli that are conveyed from the small diameter afferent fibres. The receptive fields of NS neurones tend also to be smaller than the WDR neurones which have larger receptive

fields. Still, these two types of nociceptive neurones subserve to indicate the intensity and location of a noxious stimulus (Lund and Sessle 1994)

All the information from the medullary dorsal horn projects in part directly to the thalamus or some inputs involve multisynaptic pathways that relay in the reticular formation and adjacent brainstem regions. The ventrobasal thalamus, the ventroposterior nucleus, and part of medial thalamus are the thalamic regions that are involved in transmitting nociceptive information (Hannam and Sessle 1994). The ventrobasal thalamus comprises WDR and NS neurones also and its projection to the somatosensory cortex indicates that these neurone types might have a role in pain localization and discrimination. Those neurones in the medial thalamus project to the frontal cortex and limbic system and have properties involved with pain, but more with the affective aspects of pain. Meanwhile, some information may be relayed to other parts of the nervous system including the reticular formation and the cranial nerve motor nuclei which contribute to autonomic and muscle reflex response to craniofacial noxious stimulation (Sessle 1999a).

Before understanding the mechanism of referral of pain, it is fundamental to understand the phenomena of convergence. When primary afferent neurones enter into the central nervous system they synapse with second order neurones which carry the impulses to higher centres. When diverse primary afferent neurones synapse with one second order neurone, neural information travelling along these separate primary afferents converge onto the single second order neurone. This is called “convergence”, and it helps to explain why sometimes the site of pain can differ from the source of the pain. Primary afferent neurones also can branch and synapse with several second-order neurones, and there is the possibility therefore that the pain can be referred from

one site to several remote regions, and one example of this is radiating pain (Aghabeigi 2002). TMD patients very commonly present these features of spread and referral of pain with poor localization, and this is thought to be at least partly due to convergence and divergence as well as the exchange of afferent information between the trigeminal and other craniofacial nerves via interneurones (Lund and Sessle 1994).

With repetitive or strong noxious stimulation of the tissues either through injury or through disease states, nociceptors are often sensitized to stimuli. The excitation threshold of nociceptors drops such that even usually innocuous stimuli can now activate the fibres, and silent nociceptors become excitable by both innocuous and noxious stimuli (Schaible and Richter 2004). This increased excitability of the nociceptive terminal is called peripheral sensitization (Basbaum and Jessell 2000; Basbaum and Woolf 1999; Fields 1987). The nociceptors become more sensitive to subsequent noxious stimuli (hyperalgesia) by lowering their threshold and even innocuous stimuli can provoke pain, which is referred as allodynia (Merskey and Bogduk 1994). This change in nociceptor sensitivity occurs after tissue injury or with chronic pain conditions (Aghabeigi 2002). One potential role of peripheral sensitization is as a defence mechanism for protecting the injured tissue from further injury.

If the stimulation of the first-order neurones lasts long enough, there will be persistent nociceptive input to the second-order neurones at the spinal tract nucleus or to higher-order neurones at central levels. The activation of nociceptors triggers a cascade of events in the central nervous system (CNS) together with functional changes in the spinal cord and brain that has been named central sensitization, and which may result in persistent pain (Fields 1987). This change in the processing of neurones in the medullary dorsal horn, thalamus and higher centres is a special form of neuroplasticity

(Aghabeigi 2002; Lund and Sessle 1994; Mense et al. 2001) – See also section 1.4.10 above. When the second-order neurones become more sensitized, they can respond to even innocuous stimuli (*e.g.,* light touch to skin which usually is not painful) or may spontaneously produce action potentials because of the continued production of excitatory neurotransmitters in the synapse (Okeson 1995) – See figure 1-19.

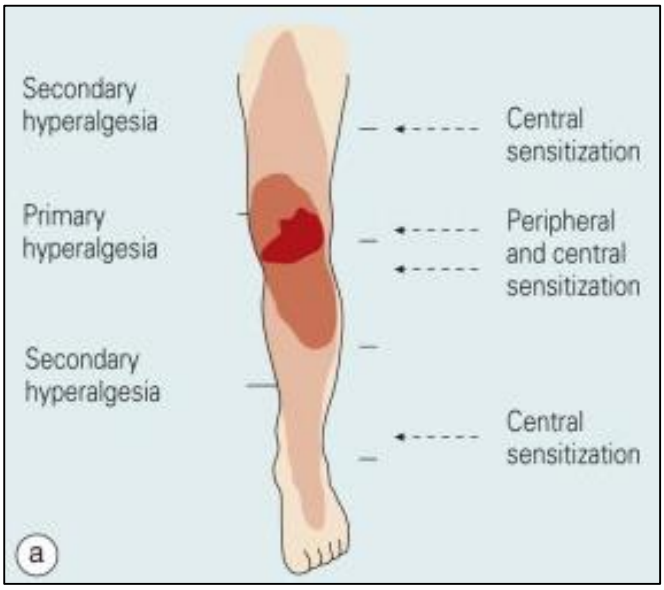


Figure 1-19: Manifestations of central sensitization. Primary and secondary hyperalgesia produced by peripheral and central sensitization. From: (Schaible 2015).

Central sensitization can result from peripheral sensitization but also from pathological discharges in afferent nerve fibres (Schaible and Richter 2004). While this may occur within the thalamus and cortex, research has been primarily focused on the dorsal horn of the spinal cord. Electrophysiological and anatomic studies have demonstrated a change in the activity and responsiveness of dorsal horn neurones in response to persistent painful stimulation. It consists of the following phenomena:

(a) Increase of the responses to input from the injured or inflamed region;

(b) Increase of the responses to input from regions adjacent to and even remote from the injured/inflamed region, although these areas are not injured/inflamed;

(c) Expansion of the receptive fields of the spinal cord, i.e. the total area from which the neurone is activated, is enlarged (Schaible and Richter 2004; Sessle 2000; Woolf and Salter 2000).

Furthermore, when the nociceptors in cutaneous tissues or in deep muscle structures are activated, the second-order neurones could continue to be sensitized longer than the stimulation itself (Lund and Sessle 1994). When these neurones are sensitized for a long period and, therefore, this neuroplasticity is prolonged, nociceptive input from the first-order neurones may no longer be essential to result in the experience of pain. This could be part of the explanation why chronic pain patients often suffer from pain despite the absence of any obvious tissue damage (Okeson 1995).

In order to have a better understanding about the chronic pain condition, it is necessary to understand how these changes in threshold and response properties of both nociceptive neurones at the injured site and in the trigeminal brainstem play a crucial role in allodynia, hyperalgesia, radiation and referral of pain. However, the cause of pain is still not clearly understood and treatment options are often not based on the cause of the pain.

1.8 Experimentally induced muscle pain

Exogenous and endogenous algesic substances have been widely used to induce experimental muscle pain in humans in an attempt to understand pain mechanisms and effects – see table 1-2 below.

Substance	Dose	Volume (mL)	Concentration	Infusion time
Hypertonic saline	0.06–7.1 mmol	0.1–10	0.3–3.4 M	Bolus–30 min
	m. abductor digiti minimi, biceps brachii, brachioradialis, deltoid, digastric, erector spinae, extensor carpi radialis brevis, extensor carpi ulnaris, extensor digitorum longus, first dorsal interosseous, flexor carpi radialis, gastrocnemius, gluteus medius, infraspinatus, longissimus, masseter, multifidus, pterygoid, quadriceps, rectus femoris, soleus, sternocleidomastoid, supinator, temporalis, tibialis anterior, trapezius, triceps brachii, vastus lateralis, vastus medialis			
	(I, II, IV, VI, VII, IX, 78, 79, 81–97, 99–101, 104–215)			
Hypotonic saline	3–27 μmol	0.2	17–137 mM	Bolus
	m. gluteus medius			
	(89, 91, 116, 117)			
Capsaicin	33–330 nmol	0.02–2	330 μM–1.6 mM	Bolus–13 min
	m. biceps brachii, brachioradialis, masseter, tibialis anterior			
	(VIII, 43, 205, 212, 216–225)			
Glutamate	20–800 μmol	0.2–0.5	0.1–2 M	Bolus
	m. masseter, splenius, tibialis anterior, trapezius			
	(182, 226–232)			
Acidic buffers	–	0.1–6.8	pH 3.2–6.3	Bolus–10 min
	m. biceps brachii, extensor digitorum brevis, flexor carpi radialis, first dorsal interosseous, pectoralis major, soleus, temporalis, tibialis anterior, triceps brachii			
	(80, 90, 120, 233–243)			
Miscellaneous	Serotonin (142, 244–246)			
	Bradykinin (142) + serotonin (90, 244, 247, 248)			
	Histamine + prostaglandin E_2 + bradykinin + serotonin (249)			
	Adenosine triphosphate (90, 249)			
	Potassium (120)			
	Calcitonin gene-related peptide + substance P or neurokinin A (250)			

Table 1-2: Algesic substances commonly used for induction of experimental muscle pain. From: (Graven-Nielsen 2006).

The technique of inducing muscle pain with hypertonic saline has been used since the 1930s and this solution has been by far the most used chemical to induce muscle pain worldwide. This is probably due to its safety and lack of side effects reported in the literature (Jensen and Norup 1992).

Thus, the intramuscular injection of 4–6% sodium chloride in volunteers is a widely used method to induce human muscle pain not only in the face region but in different

parts of the body (Graven-Nielsen et al. 1997b; Graven-Nielsen et al. 1997d). It is also worth mentioning that the solution injected (5% hypertonic saline) is only a higher concentration of normal saline that is routinely injected into individuals on a daily basis in hospitals throughout the world.

It is important to mention that a recent review of human experimental studies demonstrated that there was no evidence of asymptomatic participants developing TMD following the introduction of an experimental intervention with occlusal interference (Le Bell et al. 2002). Also, even though the differences between short-term experimental pain and chronic pain patients would be considered a limitation of this study (See section 7 – LIMITATIONS), there are sufficient similarities between experimental pain and chronic pain that should allow the generation of data and hypotheses for future studies of chronic pain patients and make inferences about the possible mechanisms involved in longer term pain.

Louca et al. showed that hypertonic saline-induced myalgia results in levels of muscle biomarkers similar to those found in the muscles of chronic myalgia patients (Louca et al. 2014). Furthermore, other features of the pain, such as McGill Pain Questionnaire pain descriptors, pain intensity and the presence of referred pain reported for experimental jaw muscle pain (Sae-Lee et al. 2008b), are the same or similar to these features identified in chronic pain patients (Graven-Nielsen and Arendt-Nielsen 2003; Gustin et al. 2011; Sae-Lee et al. 2008b).

The big advantage with experimental studies is the fact that it allows us to control carefully the intensity, duration, and modality of the noxious stimulus (Poulsen et al.

1995), whereas clinical studies with long-term pain patients can be easily confounded by many factors (e.g. cognitive, emotional, and social factors) that make it difficult to look at specific aspects of the disease. Therefore, it is hoped that these studies will generate important baseline information and hypotheses for future clinical studies.

1.9 EMG recording methodology:

There are two main kinds of EMG electrodes that have been described in the literature: needle (or intramuscular) electrodes and surface electrodes. Intramuscular electrodes are inserted into the muscle and therefore it is usually easier to detect single motor unit activity that is the activity of one or more single motor units. However, the discomfort from the needle insertion procedure is a factor in participation in these experiments. Another limitation of the intramuscular electrodes is that once the needle is placed, the only way to change the location of the electrode is by pulling the fine wires towards the skin (Duchateau and Enoka 2011).

Surface electrodes, on the other hand, are easier to place and remove than intramuscular electrodes, are more comfortable and have the advantage of providing a signal that is more representative of the activity from the whole muscle rather than one specific spot within a muscle as is provided with intramuscular electrodes (Ahlgren et al. 1980). However, the background noise present with surface EMG recordings together with the large area over which the surface electrodes usually pick up activity from many motor unit action potentials, means that surface EMG recordings usually do not permit the discrimination of single motor units. This inability to discriminate single motor units with surface EMG recordings applies especially to the small single

motor units which are usually the first recruited in a motor action. In addition, surface EMG is usually unsuitable for recordings from deep muscles or muscles exhibiting low levels of activity (Duchateau and Enoka 2011).

A history and clinical evaluation allows a diagnosis of TMD to be made, although it does not usually explain the causes of TMD, nor does it always make a significant contribution to developing a therapy plan. Assuming that EMG could aid in the diagnosis of TMD by assessing the electrical characteristics of the muscles and specific muscular dynamics, it is reasonable to suggest that it could possibly be used to monitor the effect of the therapy that is being used to detect subclinical TMDs, or even prevent this pathology before the beginning. However, the use of EMG in the diagnosis and management of orofacial pain, and TMD conditions in particular, has not been well substantiated in the literature, and it is important to mention that, at the present state of knowledge, EMG should never replace clinical examination (Gonzalez et al. 2008; Lund et al. 1991; Reid and Greene 2013). The use of EMG measurements for diagnostic purposes has low reliability and validity due to technical issues or different factors such as electrode placement, electrode position, inter-electrode distance, cross-talk, head or body movement by the person, or existing pain conditions and data interpretation problems (Gonzalez et al. 2008; Klasser and Okeson 2006).

In fact, to distinguish real changes from biological and instrumental noise, standardization of the EMG recording technique itself including parameters should be recommended. Therefore, researchers have investigated the relationship between orofacial motor function and pain by means of EMG analysis, in standardized research

settings, and therefore by minimizing the effects of confounding factors Svensson and Graven-Nielsen 2000.

Despite the advance in the area, until this point, no specific criteria or cut off point have been reported that can discriminate, with adequate sensitivity and specificity, between healthy individuals and those with TMD (Gonzalez et al. 2008; Lund et al. 1995).

1.10 Masseter and temporalis muscles

The four principal muscles of mastication are the medial and lateral pterygoid muscles, and the temporalis and masseter muscles- See table 1-3 and figure 1-20; their actions produce movements of the mandible around the temporomandibular joints.

MUSCLE	ORIGIN	INSERTION	MAIN ACTIONS
Temporalis *	Floor of temporal fossa and deep temporal fascia	Ramus of mandible and coronoid process	Elevates mandible; posterior fibers retrude mandible
Masseter	Zygomatic arch	Ramus of mandible and coronoid process	Elevates and protrudes mandible; deep fibers retrude mandible
Lateral pterygoid	*Superior head* : infratemporal surface of greater wing of sphenoid *Inferior head* : lateral pterygoid plate	Pterygoid fovea, capsule of TMJ, articular disc	Acting together, protrude mandible; acting alone and alternately, produces side-to-side movements
Medial pterygoid	*Deep head* : medial surface of lateral pterygoid plate and palatine bone *Superficial head* : tuberosity of maxilla	Ramus of medial mandible, inferior to mandibular foramen	Elevates mandible; acting together, protrude mandible; acting alone, protrudes side of jaw; acting alternately, produces grinding motion

Table 1-3 : Summary of the muscles of mastication, their origins, insertions and main actions. From (Hansen 2014).

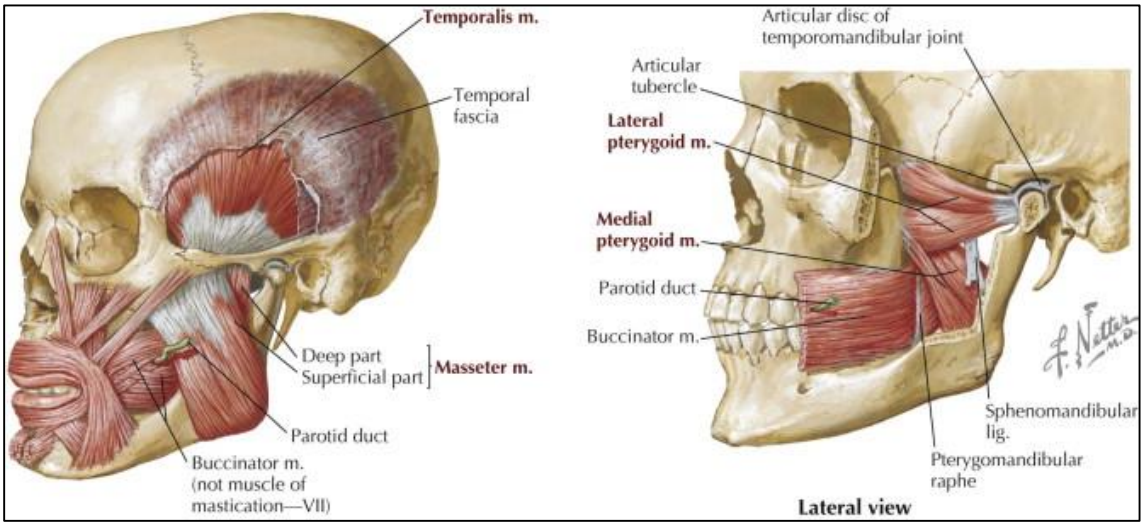


Figure 1-20: The masseter and temporalis muscles on the left side of the head. The lateral pterygoid and medial pterygoid muscles are also shown. From: (Hansen 2014)

A detailed discussion regarding the masseter and temporalis muscles is in the next sections (1.10.1 and 1.10.2).

1.10.1 The temporalis muscle

The temporalis muscle arises from the whole of the temporal fossa up to the inferior temporal line and from the deep surface of the temporal fascia. The muscle is covered by two fascia layers (Ahmed and Risal 2013) – See figure 1-21.

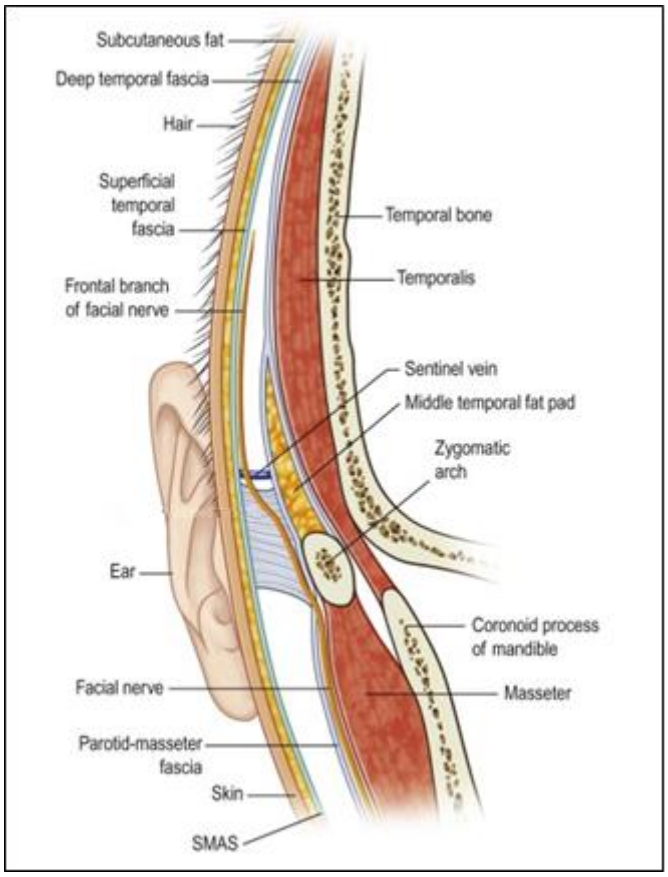


Figure 1-21: The facial layers of the temporal region. From: (Ahmed and Risal 2013). SMAS: superficial facial fascia.

The temporalis fibres arise from the temporal fossa and the deep part of temporal fascia descend and converge into a tendon that passes through the gap between the side of the skull and the zygomatic arch. A temporal fascia is attached to the superior surface of the zygomatic arch, and covers the muscle. Using a horizontal section above the zygomatic bone, the muscle would be represented in a triangular shape, with a broad base at the front and a long-running tip at the rear (Schumacher 1961; Susan Standring M.B.E. et al. 2016).

The temporalis muscle – figure 1-22 - is attached to the medial surface, apex, anterior and posterior borders of the coronoid process and to the anterior border of the

mandibular ramus nearly down to the third molar tooth. Its anterior fibres are oriented vertically, as opposed to the most posterior fibres that run almost horizontally and the intervening fibres have intermediate degrees of obliquity, almost like a fan – See figure 1-23 (Susan Standring M.B.E. et al. 2016). Some fibres of the temporalis muscle may sometimes be attached to the articular disc.

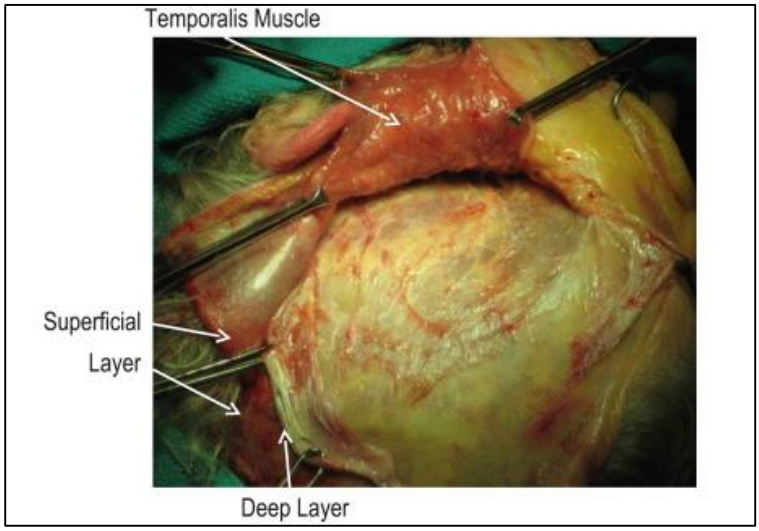


Figure 1-22: Temporalis muscle. From: (Ahmed and Risal 2013). Dissection deep the temporalis muscle. The muscle can be left as part of the skin flap. This is a safe and easy plan if no exposure of the arch is needed.

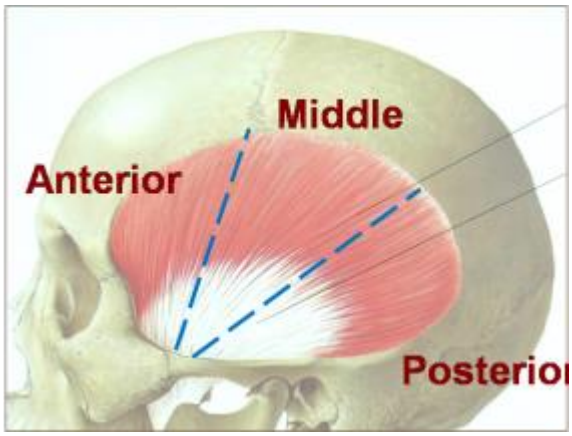


Figure 1-23: The anterior fibres of temporalis descend vertically; traced posteriorly the fibres are increasingly oblique, while the most posterior fibres are almost horizontal. From: (Reddy 2015).

Table 1-4 summarizes the vascular supply, innervations and actions of the muscle.

Vascular supply	Innervation	Actions
It is supplied by deep temporal branches from the second part of the maxillary artery, which enter on its deep aspect, and middle temporal branches from the superficial temporal artery, which enter on its lateral aspect.	It is supplied by the anterior, middle and posterior deep temporal branches of the anterior trunk of the mandibular division of the trigeminal nerve.	Temporalis elevates the mandible and so closes the mouth and approximates the teeth. The muscle also contributes to side-to-side grinding movements. The posterior fibres retract the mandible after it has been protruded. The posterior fibres of temporalis, which are almost horizontal, are one source of mandibular retrusion.

Table 1-4: Vascular supply, innervation and actions of the temporalis muscle. Table based on (Susan Standring M.B.E. et al. 2016).

Regarding the blood supply, the temporalis muscle receives its supply from the anterior and posterior deep temporal arteries. Those, in turn, arise from the maxillary artery and supply the muscle through its deep surface. The temporalis also receives a secondary blood supply from the middle temporal artery (Ahmed and Risal 2013).

1.10.2 The masseter muscle

The masseter – Figure 1-24- is a powerful masticatory muscle that elevates the mandible. It also plays an important morphological role in the lower facial contour (Lee et al. 2012). Its internal architecture has been described in rat (Nordstrom and Yemm 1974), pig (Herring et al. 1979), rabbit (Weijs and van der Wielen-Drent 1983), monkey, orang-utan and gorilla (Yoshikawa et al. 1962), seal (Naito and Naito 1973) and human (van Eijden and Raadsheer 1992).

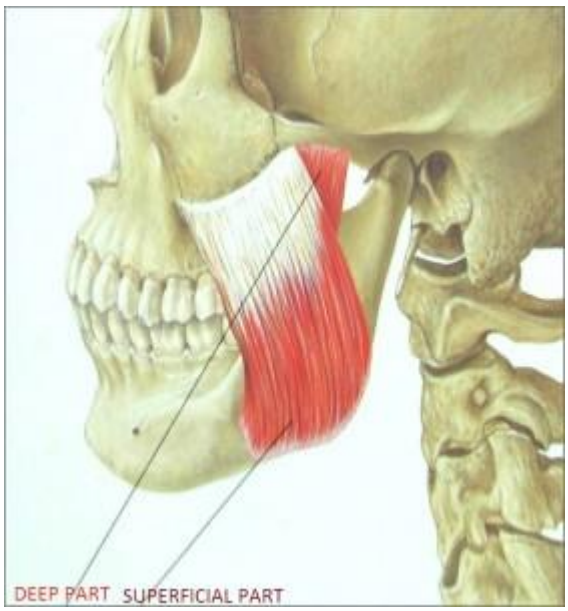


Figure 1-24: Masseter muscle, it deep and superficial lawyers. From: (Reddy 2015).

This muscle seems to consist of three layers – See Figure 1-25, the superficial masseter, intermediate masseter and the deep masseter (Gaudy et al. 2000). The superficial layer is known to be the largest. It 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. The fibres of this layer pass downwards and backwards, to insert into the angle and lower posterior half of the lateral surface of the mandibular ramus. Its superficial fibres are angled approximately 10° from the vertical, as it can be visible in lean individuals. In the coronal (frontal) plane, the masseter forms an approximately 10° angle with the mandibular ramus. The ridges on the surface of the ramus are caused by intramuscular tendinous septa in this layer. The middle layer of masseter arises from the medial aspect of the anterior two-thirds of the zygomatic arch and from the lower border of the posterior third of this arch. It inserts into the central part of the ramus of the mandible. Finally, the deep layer of this muscle arises from the deep surface of the zygomatic arch and inserts into the upper part of the mandibular ramus and into its coronoid process. The deep fibres run vertically, and are evident just anterior to the temporomandibular joint, where they are not covered by the more superficial layers (Susan Standring M.B.E. et al. 2016).

Table 1-5 summarizes the vascular supply, innervations and actions of the muscle.

Vascular suply	Innervation	Actions
Masseter is supplied by the masseteric branch of the maxillary artery, the facial artery and the transverse facial branch of the superficial temporal artery.	Masseter is supplied by the masseteric branch of the anterior trunk of the mandibular division of the trigeminal nerve.	Masseter elevates the mandible to occlude the teeth in mastication and has a small effect in side-to-side movements, protraction and retraction.

Table 1-5: Vascular supply, innervation and actions of the masseter muscle. Table based on (Susan Standring M.B.E. et al. 2016).

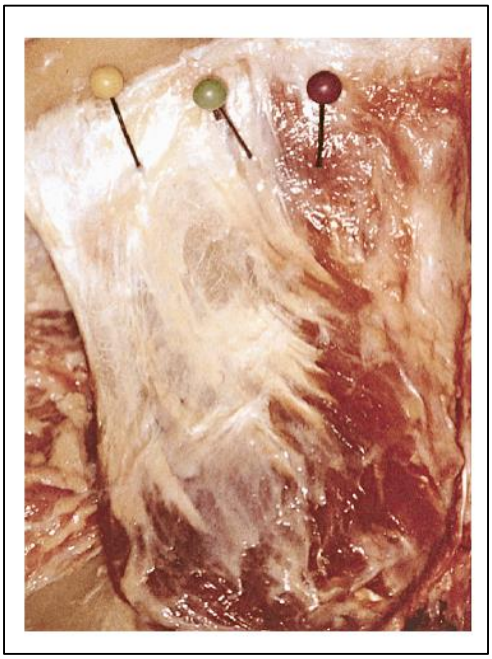


Figure 1-25: General view of masseter m. Superficial m. (yellow needle), intermediate m. (green needle), deep m. (red needle) from: (Gaudy et al. 2000).

1.11 SUMMARY

A number of theories have continued to accumulate knowledge regarding the relationship between pain and motor activity. As it was pointed out in this literature review, there are some major issues with the earlier theories that do not appear to take into account all of the observations of the interrelationships between pain and motor activity. These include that these earlier theories only operate at the brainstem level (the spinal cord or segmental level for the spinal system) and the fact that 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. Besides, these theories cannot take into account the influence of higher brain centres and psychological factors.

Surface electromyography of masticatory muscles have been made both in clinical and experimental pain conditions to analyse muscle activity and generally propose only small or no effects on jaw muscle EMG activity as a result of pain. However, there have been far fewer studies of the effects of pain on single motor unit (the basic functional unit) activity within the painful muscle.

Recent evidence in the jaw motor system provides evidence that experimental painful stimulation of the masseter muscle results in both increases and decreases in motor unit activity, in other words, a reorganization of motor unit activity during the generation of the same direction and level of force within the masseter muscle (Minami et al. 2013) and in different sites within the masseter muscle (Malik 2016). However, it is unclear

whether this reorganization occurs only within the painful muscle or whether it may also occur in other non-painful synergetic muscles. An understanding of these issues would add to our understanding of how motor unit activity within the masseter muscle and in other muscles changes in response to noxious stimulation and thereby may have implications for understanding changes in jaw muscle activity in TMD. This is very important as some management strategies for chronic orofacial pain are still based on prior simplistic models proposing uniform effects of pain on muscle activity and it may lead to the development of more reliable management strategies and more effective treatments to patients.

2 AIMS AND HYPOTHESIS

Therefore, the main question of this study was whether the pain-induced reorganization of activity that has been previously demonstrated within one jaw muscle (right masseter) also occurs in other synergetic jaw muscles (in this thesis, the right temporalis muscle).

This was investigated by recording muscle activity, in terms of single motor units, within the muscle receiving the noxious stimulus as well as in another non-painful muscle. The design of the experiment involved a comparison of the motor effects of hypertonic saline infusion (painful) with isotonic saline infusion (minimal or no pain). This comparison allowed us to conclude whether any changes observed in muscle activity are due to pain.

2.1 Aims:

1- To determine whether experimental noxious stimulation of the right masseter muscle, in comparison with control, modifies the ability of individuals to execute isometric jaw-closing tasks.

2- To determine whether experimental noxious stimulation of the right masseter muscle results in significant ($p<0.05$) changes in the activity patterns (i.e. SMU recruitment patterns and thresholds) in the right temporalis muscle (a non-

painful synergistic muscle) and right masseter muscle during isometric ramp jaw closing tasks at two different rates of force increase (slow and fast).

3- To determine whether experimental noxious stimulation of the right masseter muscle results in significant ($p<0.05$) changes in the activity patterns (i.e. SMU recruitment patterns and firing rates of single motor units, and root mean square EMG activity) in the right temporalis muscle (a non-painful synergistic muscle) and right masseter muscle during isometric 2 step-levels jaw closing tasks.

4- To determine whether experimental noxious stimulation of the right masseter muscle results in significant ($p<0.05$) changes in the activity patterns (i.e. recruitment patterns) in the right masseter (painful muscle) and in the right temporalis muscle (a non-painful synergistic muscle) that are not consistent with earlier theories of pain-motor interaction, namely, the Vicious Cycle Theory and The Pain Adaptation Model.

5- To determine whether experimental noxious stimulation of the right masseter muscle results in significant ($p<0.05$) changes in the activity patterns in the right temporalis muscle and right masseter muscle that are associated with the scores from some psychological measures.

2.2 Hypothesis:

According to the aims of the study, it is hypothesized that:

1- Experimental noxious stimulation of the right masseter muscle, in comparison with control, does not modify the ability of individuals to execute isometric jaw-closing tasks.

2- Experimental noxious stimulation of the right masseter muscle results in significant ($p<0.05$) changes in the activity patterns (i.e. SMU recruitment patterns and thresholds) in the right temporalis muscle (a non-painful synergistic muscle) and right masseter muscle during isometric ramp jaw closing tasks at two different rates of force increase (slow and fast).

3- Experimental noxious stimulation of the right masseter muscle results in significant ($p<0.05$) changes in the activity patterns (i.e. SMU recruitment patterns and firing rates of single motor units, and root mean square EMG activity) in the right temporalis muscle (a non-painful synergistic muscle) and right masseter muscle during isometric 2 step-levels jaw closing tasks.

4- Experimental noxious stimulation of the right masseter muscle results in significant ($p<0.05$) changes in the activity patterns (i.e. recruitment patterns) in the right masseter (painful muscle) and in the right temporalis muscle (a non-painful synergistic muscle) that are not consistent with earlier theories of pain-motor interaction, namely, the Vicious Cycle Theory and The Pain Adaptation Model.

5- Experimental noxious stimulation of the right masseter muscle results in significant ($p<0.05$) changes in the activity patterns in the right temporalis muscle and right masseter muscle that are associated with the scores from some psychological measures.

3 MATERIALS AND METHODS

3.1 Recruitment

Twenty participants (15 females, 5 males) between the ages of 22-40, with at least 24 permanent teeth, no missing molars and no current orthodontic treatment were recruited for this study. Exclusion criteria were the presence of any orofacial pain at the time of the experiment, diagnosis of TMD, neurological disorders (e.g. cerebral palsy, Parkinson's disease), bleeding disorders, cardiac problems, current pregnancy, high blood pressure, presence of systemic musculoskeletal pain disorders (e.g. fibromyalgia, inflammatory joint disease), serious systemic disease (e.g., current malignancies), medications for chronic diseases or medications that might influence muscle activity or a response to pain.

Participants were recruited from the students of the University of Sydney, the general public, and family and friends of the research investigators. All potential participants were invited to take part in the study if they met the inclusion/exclusion criteria and were informed that participation was voluntary and that they were under no obligation to participate.

Detailed information about the research and possible complications during and after the experiment were also explained to potential participants before they were invited to take part in the study. Participants were asked to sign a written informed consent form before being enrolled in the study and were also informed again about their right to withdrawn from the experiment at any point without the need to provide any explanation. The study was conducted in three sessions, all of which were located at

the Jaw Function and Orofacial Pain Research Unit based at Westmead Centre for Oral Health, Sydney, Australia. Sessions 1 and 3 involved the participant to be present, while session 2 involved customized construction of the bite force device.

The study was approved by the Human Research Ethics Committee of the University of Sydney.

3.2 First session

For the first session, a clinical and psychological assessment for the investigation of the presence of TMD was made with the Research Diagnostic Criteria for Temporomandibular Disorders (RDC/TMD) (Dworkin and LeResche 1992) – see Appendix 8.1 - by a single calibrated examiner. When the absence of signs or symptoms of TMD was confirmed, alginate impressions of the upper and lower teeth were made in order to construct plaster cast models to facilitate the construction of the intraoral splints to house the bite force transducer. The impressions were poured in dental stone (Yellowstone, Lordell Trading Pty. Ltd.).

3.3 Second session

3.3.1 Splint

With the participant's plaster cast models, a thermoforming foil disc (Erkodent, Erkoplast-0, 1.5 mm thickness and 120 mm diameter) was used to produce a custom-

made polyvinal splint that covered the upper teeth much like a very thin mouthguard (see Figure 3-1). Another polyvinal splint was made for the lower teeth using the same methods as above (see Figure 3-1).

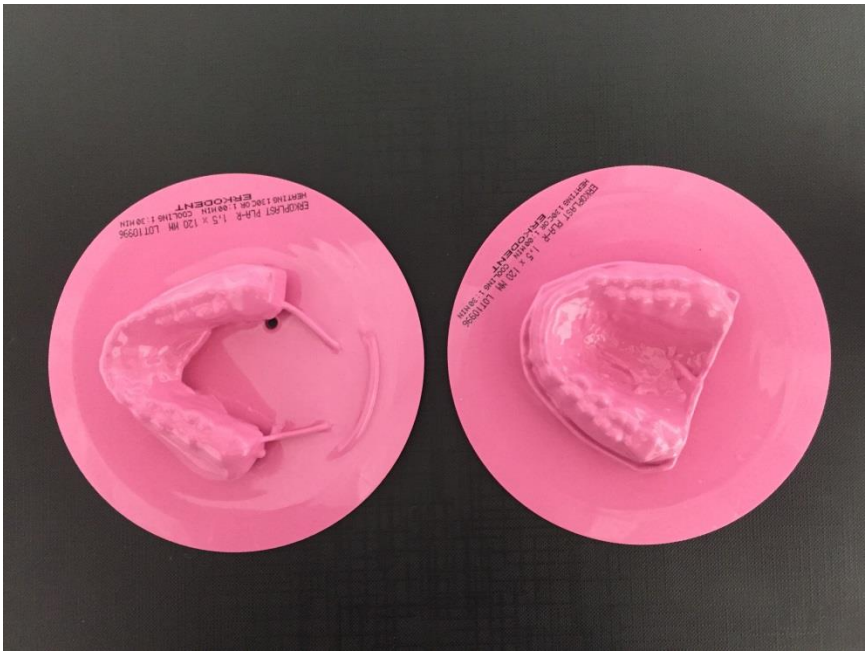


Figure 3-1 : Custom-made polyvinal splints before being trimmed. Upper teeth on the left of the panel and lower teeth on the right of the panel

Because the material's chemical characteristics allows it to bond to polymethyl methacrylate, the upper splint was modified with the use of acrylic resin to accommodate a metal housing for a small force transducer (LMA-A; Kyowa Dengyo, Tokyo, Japan) to measure vertical jaw closing force (see Figure 3-2). The lower splint was also modified by adding acrylic resin to house a small plate (lower plate, Figure 3-2) that was oriented parallel to the occlusal plane. The force transducer and housing were embedded in acrylic which was secured to the upper splint (Figure 3-2). A hinged plate contacted the force transducer sensor and on the lower part of this hinged plate an acrylic ball was secured. The ball provided the contact with the lower plate secured to the lower acrylic splint. This arrangement was designed so that when the participant produces a vertical force, the force transducer would record the amount of force

applied (see Figure 3-2), and that non-vertical forces would result in slippage of the ball along the lower plate.

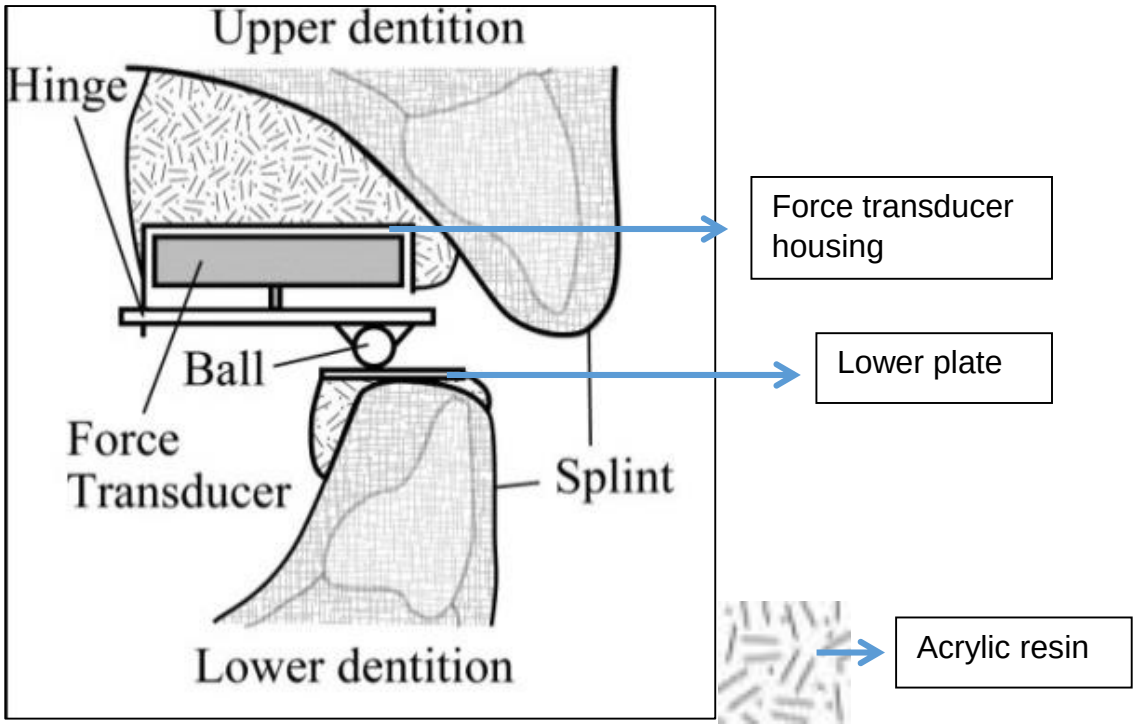


Figure 3-2: Bite force transducer in a lateral view. The upper and lower splints accommodate the housing for the force transducer and the lower plate respectively (Reproduced with modifications from (Minami et al. 2013).

The casts were initially articulated on a semi-adjustable articulator (Denar Mark II, whip mix, Louisville, KY 40217 USA) at the intercuspal position and at an average face-bow position (Figure 3-3). To enable set-up of the force transducer on the acrylic splints, the articulator was opened until its maximum, and the force transducer was secured in place via acrylic on the customised splints.

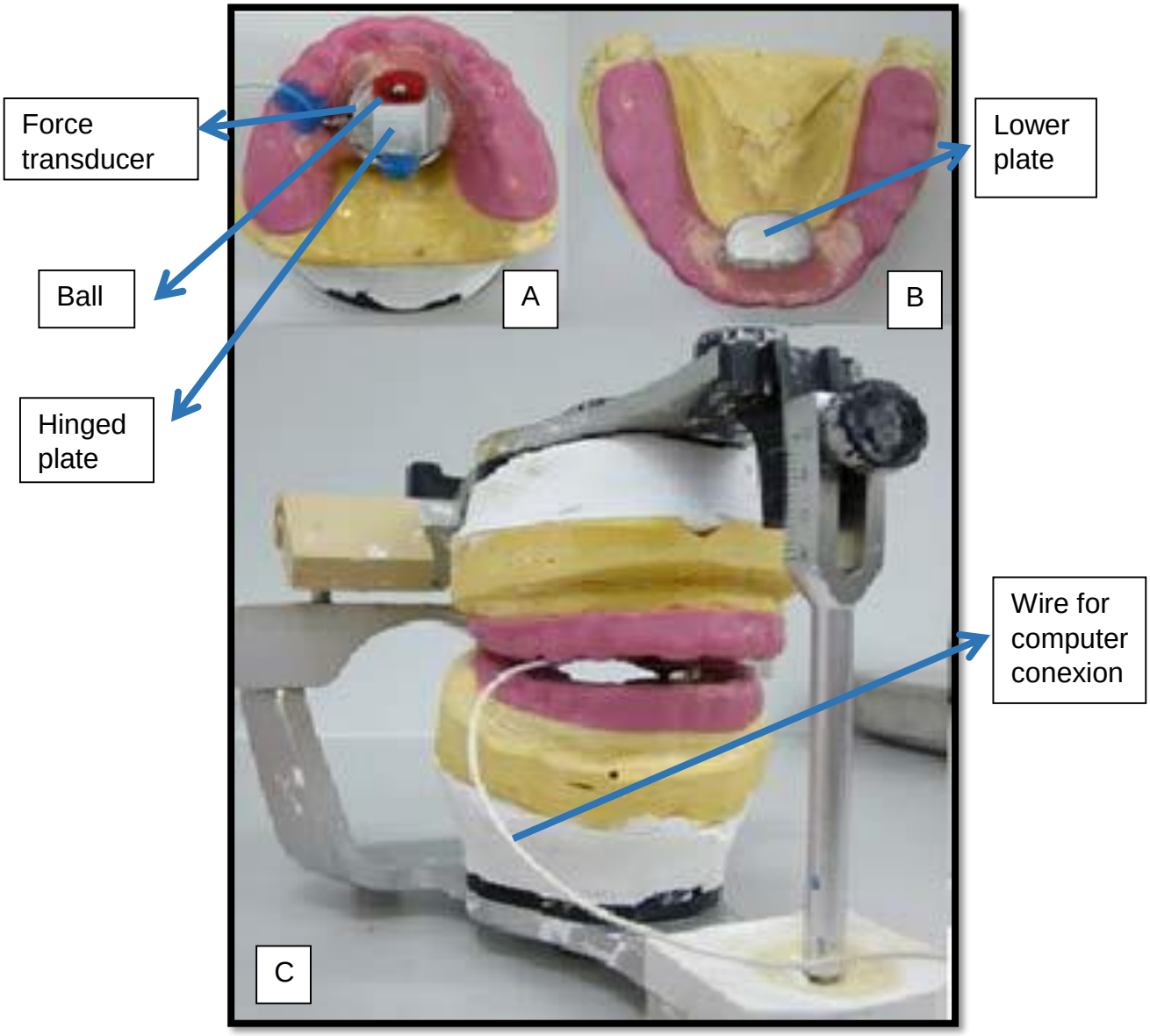


Figure 3-3: Polyvinal splints after customization and adaptation of the force transducer holder and lower plate. Reproduced with modifications from (Malik 2016). A: Upper cast model with upper splint. B: Lower cast model with lower splint. C: Upper and lower cast models articulated.

3.3.2 Electrode construction

Two Teflon stainless-steel fine wires (Teflon®-coated stainless steel wire - diameter 0.0045 mm) were threaded through a disposable spinal needle (24-26 gauge needles - 25mm long). The two fine wires were then bent at the end of the needle (~3-4mm out

of the needle), and one of the wires were 2 mm shorter than the other to minimize the chances that the two wires would touch once inserted into the muscle. As Teflon surrounded the wires, the end of each wire was scraped to remove the Teflon at around 0.5 mm from the end, in order to expose bare wire. The other end of the wire, were also scraped at a longer part (~10 mm) in order to connect with the recording apparatus on the day of the experiment.

Figure 3-4 shows the needle with two separated fine wires.

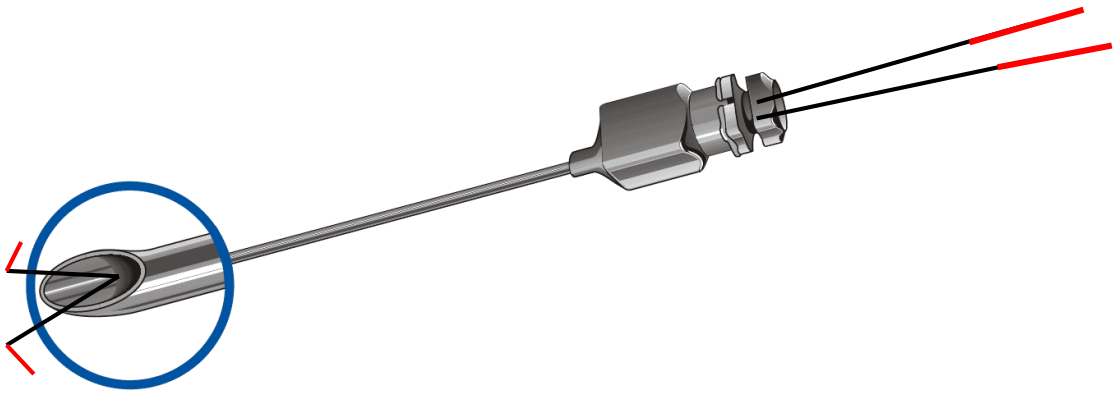


Figure 3-4: Needle with two stainless-steel fine wires. Blue circle indicates the separation of the wires which were bent at the end. Red lines show where the Teflon was removed to expose the bare wire.

3.4 Third session

The third session of the experiment is summarized on the table 3-1 below:

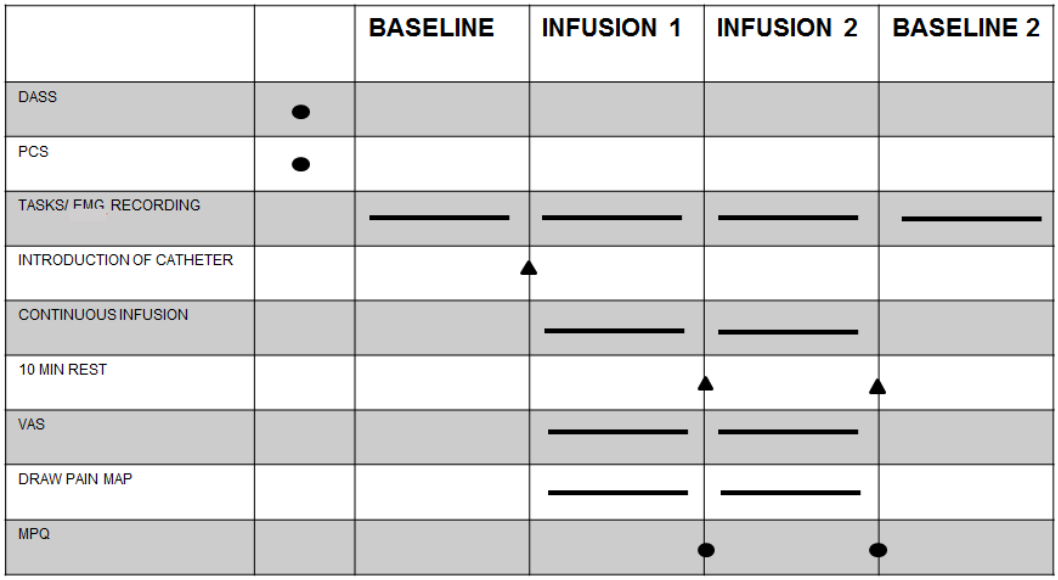


Table 3-1: Schematic sequence of the experiment. Circles: moment where some questionnaire was applied (DASS, PCS, MPQ). Triangles: events that happened between blocks; namely, introduction of catheter between the baseline and infusion 1, and 10 min rest between infusion 1 and infusion 2, and between infusion 2 and baseline 2). Lines: activities performed throughout the blocks.

3.4.1 Health assessments and questionnaires:

Firstly, details of the participant's medical history and current health state were obtained with the use of a screening questionnaire on the day of the experiment to confirm their eligibility to be part of the study - see Appendix 8.2

Two additional questionnaires were completed to clarify possible associations between motor function and psychological status (Aim 5). Therefore, on the day of the experiment, but prior to starting the experiment, the participants were asked to complete the Depression, Anxiety and Stress Scales 21 (DASS21) (Lovibond and Lovibond 1995a) (Appendix 8.3) and the Pain Catastrophizing Scale (PCS) (Sullivan MJL 1995) (Appendix 8.4).

The DASS21 questionnaire is a reliable and well validated self-report measure of the three negative emotional states of depression, anxiety and stress (Crawford and Henry 2003; Lovibond and Lovibond 1995b). The instrument consists of 21 statements where the participant was required to mark on a 4-point Likert scale how much each statement applied in the past week. The scale ranges from “Did not apply to me at all” to “Applied to me very much, or most of the time”. The total scores for each scale were calculated by the summation of the 7 items that relate to the emotional states. The higher scores on each scale indicate higher risk of depression, anxiety or stress. The Depression scale assesses hopelessness, low self-esteem, and low positive affect. The Anxiety scale assesses autonomic arousal, physiological hyper-arousal, and the subjective feeling of fear. The Stress scale items assess, tension and agitation (Lovibond and Lovibond 1995b).

Pain catastrophizing is the exaggerated negative belief about a painful experience which may influence the participant’s perception of pain intensity (Turner et al. 2002). The PCS is a 13 statement questionnaire which asks the participant to rate on a 5-point scale ranging from “not at all” to “all the time”, the degree to which each statement applies to them (e.g., “I keep thinking of other painful events”; “I felt I was close to

panic") in terms of the thoughts the participant have when they are in pain. The PCS total score was computed by summing responses to all 13 items that compose this questionnaire. The PCS total scores range from 0 – 52. The PCS subscales are computed by summing the responses to the following items:

Rumination: Sum of questions 8, 9, 10, 11

Magnification: Sum of questions 6, 7, 13

Helplessness: Sum of questions 1, 2, 3, 4, 5, 12

The total score (ranging from 0-52) indicates the level of pain catastrophizing (Sullivan MJL 1995).

3.4.2 Electrode placement and EMG recording

After the completion of the DASS-21 and PCS, each participant was seated in a dental chair in a quiet room. The skin overlying the right and left masseter and right and left temporalis muscles was cleaned with the use of alcohol pads (70% isopropyl alcohol, Alco wipe, Pro medica, Australia) and then disposable bipolar surface EMG electrodes (4 mm × 7 mm recording area, Duo-Trode, Myotronics, Washington, USA) were attached with non-allergenic adhesive tape (Transpore™ Surgical Tape, 3M™, St. Paul, MN, USA) to the skin overlying these muscles.

A small amount (1-2 mL) of electrode conducting gel (Sigma Gel, Medtronic Denmark) was injected just under the conducting foam via hypodermic needle in order to facilitate the recording of the surface EMG signal. The area for the placement of the surface electrodes was chosen by palpating the muscles for identification of the most active

area during contraction and the electrodes were oriented parallel to the direction of the main fibres of the muscles. These surface electrodes were placed to help confirm that the tasks were being performed correctly by the participants, and also the surface EMG data were acquired for future analysis. The surface EMG data were not analysed in this thesis as the data were described as part of another parallel study (Sandoval, 2017).

The 2 splints were then inserted into the participant's mouth. Subsequently, bipolar fine-wire electrodes were placed at 1 location in the right masseter and 1 location in the right temporalis muscle. Prior to the placement, the participant voluntarily contracted the masseter and temporalis muscles by repeated clenching activities. The masseter muscle contraction was palpated to identify the borders of the muscle in order to place the fine wire electrodes horizontally and vertically mid-muscle. The temporalis was palpated to identify the region 1cm posterior of the anterior border for placement of the fine wire electrodes.

About 20 minutes before the insertion of the intramuscular electrodes, a topical anaesthetic gel (Emla, Astrazeneca, Australia) was applied on the skin over the right masseter and right temporalis areas that had previously been identified and this minimized the discomfort caused by the introduction of the needle.

Intramuscular electrode placement was made via 24-26 gauge needles (25-mm-long) (Sae-Lee et al. 2006) containing 2 fine wires that were bent back over the tip of the needle 2-5 mm from the ends of the wires. The needle was inserted in a standardized inclination and was withdrawn leaving the fine-wire electrodes in the muscle. For each intramuscular electrode insertion, the needle was inserted ~20 mm beneath the skin

or until the needle contacted bone. For the masseter intramuscular insertion, the needle was angled downwards at ~30 degrees in relation to the ramus. For the temporalis muscle insertion, the needle was angled downwards at ~45 degrees relative to the horizontal. After insertion, the needle was withdrawn, leaving the fine wires within each muscle. The electrodes were sterilised prior to placement in each muscle.

The EMG activities from the intramuscular and surface electrodes were confirmed prior to the experiment by asking participants to clench their teeth in order to confirm placement and that all the acquisition equipment was working satisfactorily. A ground electrode was attached to the left wrist.

The EMG activity was amplified with a bioelectric 1902 isolated pre-amplifier (Cambridge Electronic Design Limited, Cambridge, UK), with an amplification ranging from 3,000 to 10,000x for the intramuscular electrodes according to the intensity of the EMG signal displayed on each muscle channel on the computer screen.

The EMG signal was digitized by CED Micro 1401-3 processor (equipment for data acquisition from Cambridge Electronic Design, Cambridge, UK) for subsequent offline analysis (Figure 3-5). Muscle activity from the intramuscular electrodes was sampled at a bandwidth of 20 Hz to 2.5 kHz in the first 5 participants at a sampling rate of 5,000 samples/s. For the remaining 15 participants, the muscle activity from the intramuscular electrodes was sampled at a bandwidth of 20 Hz to 10 kHz at a sampling rate of 20,000 samples/s. As the force data were only used to choose a maximum and a minimum force and the average rate of change of force between these forces, as well as establishing a stable force level, then a lower sampling rate for the remaining participants was considered to be adequate. The EMG muscle activity was recorded

synchronously with the force output from the force transducer.

During every experiment, Spike2 software (Cambridge Electronic Design Limited, Cambridge, UK) was used to provide visual feedback, on a computer screen placed in front of the participant, of the level of the force in volts that was being exerted. The force output was displayed on the computer screen as a horizontal line.

The horizontal line displaced in the positive direction on the y-axis with increases in closing force. Scripts written with Spike2 software were used to generate a target line that was superimposed over the force transducer output (see below for detailed description of the tasks). Participants were instructed to match the force line with the target levels as closely as possible. The scripts controlled the rate at which the force changed for all the tasks.

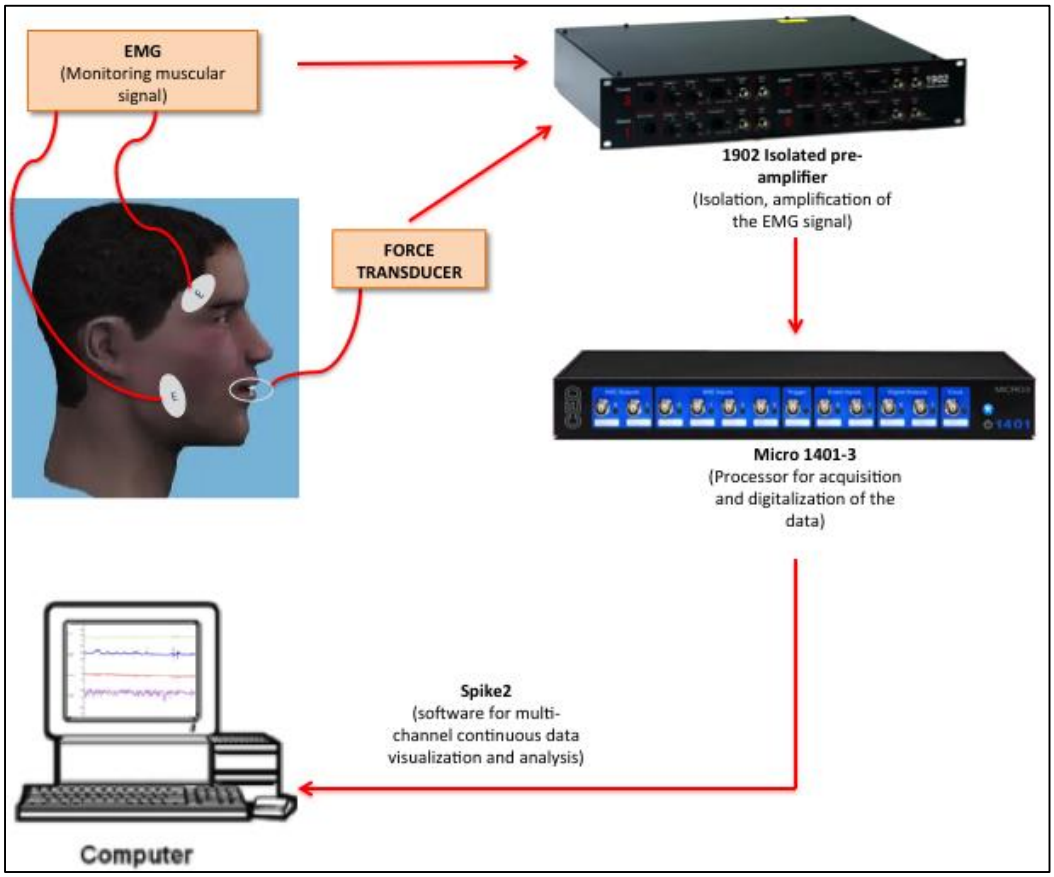


Figure 3-5: The EMG signals from the temporalis and masseter muscles were amplified by the 1902 Isolated pre-amplifier and the Micro 1401-3 for acquisition and digitization of the EMG muscle activity. The Spike2 was the software used for data acquisition, visualization and analysis. Reproduced from: (Sandoval 2017).

3.4.3 Tasks

The participant was asked to perform 3 different isometric jaw-closing tasks (clenching tasks) using the bite force transducer constructed in the second session. Each performance of a task was termed a 'trial'. Every trial commenced with a rest period of 2-3 s prior to the initiation of the increase in closing force associated with the tasks – See figure 3-7.

1. A **slow ramp jaw closing task** that involved increasing jaw closing force at a low force rate (of 5 N/s). This task took 18.5 s for completion of the task, excluding the initial 2-3 s rest period prior to the initiation of the increase in force.

2. A **fast ramp jaw closing task** that involves increasing jaw closing force at a higher force rate (of 17 N/s) than for the slow ramp jaw closing task. This task took 7 s for completion of the task, excluding the initial 2-3 s rest period prior to the initiation of force increase.

3. A **2 step-levels jaw closing task** where the participant increased jaw closing force up to a first level (step 1), held for 3 s and then, increased the force up to a second level (step 2) and held again for another 3 s before relaxing. Both levels were customised for each participant based on the visual confirmation on the computer screen of the recruitment of the first single motor units as the determination of the force level that will constitute step 1 for that participant. Then, for step 2, the participant increased closing force so that there was a clear increase in the firing rates of the existing single motor units and/or there was recruitment of additional single motor units (See figure 3-6). Both of the closing forces levels considered were readily achievable closing forces. The step task took ~8 s for completion of the task excluding the initial 2-3 s period prior to the initiation of force increase; there was a duration of 0.5-1 s for the increase in force from rest to step 1, and from step 1 to step 2.

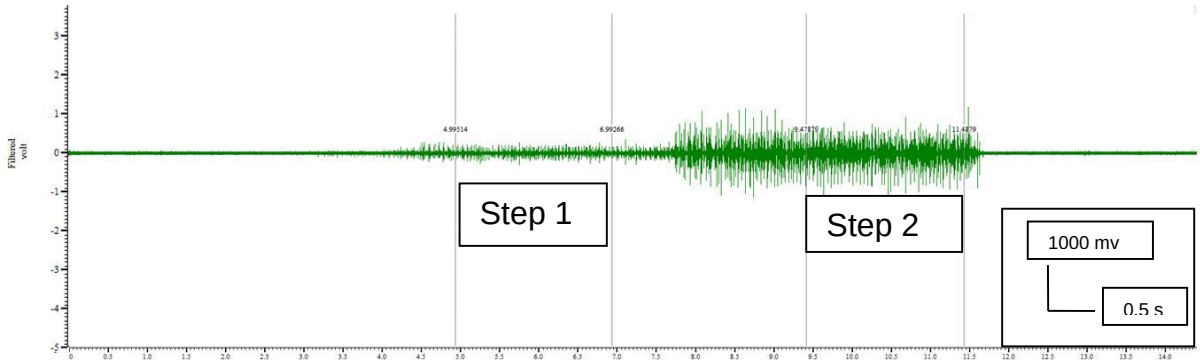


Figure 3-6: Increased force showing the difference between step 1 and step 2. X-axis: time in seconds (Sec). Y-axis: force transducer output in volts (V).

For the 2 step-levels jaw closing task, once established, the same customised force levels were used until the end of the experiment for that specific participant in order to standardize the step levels during the whole experiment.

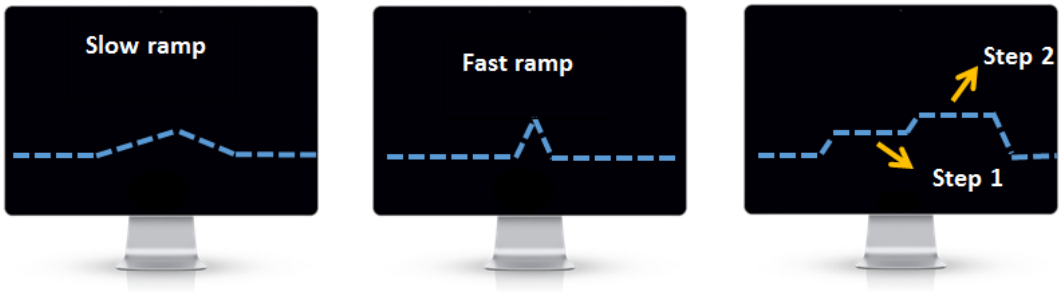


Figure 3-7: Schematic figure of target forces on the computer screen placed in front of the participant and which each participant was instructed to match to achieve the same force rates and levels in every trial.

Each of the three isometric jaw closing tasks was repeated 3 times, that is 3 trials were performed for each task. These 9 trials were then repeated over 4 blocks, which were:

1- Block 1: baseline, before any infusion
2- Block 2: infusion of hypertonic saline or isotonic saline infusion (the order was alternated between participants)
3- Block 3: infusion 2, after isotonic saline or hypertonic saline infusion (the order was alternated between participants)
4- Block 4: baseline 2, after the two infusions were completed.

If technical difficulties were encountered, extra repetitions were needed due to technical issues (e. g. when the EMG signal was capturing lots of noise). In all trials, participants were instructed and motivated to match bite force as closely as possible to the target force levels marked for that participant. The electrodes were left *in situ* for approximately 3 hours.

3.4.4 Induction and assessment of jaw-muscle pain

The induction and assessment of jaw-muscle pain followed closely our previously published protocol (Sae-Lee et al. 2006). Firstly, a disposable 22 or 24 gauge needle integrated IV catheter (Smiths Medical, Lancashire, UK) was placed into the mid-portion of the right masseter muscle until bone was reached (Sae-Lee et al. 2006; Stohler et al. 1992). A 1 ml syringe (Becton Dickinson, Singapore) was then attached to the catheter before the injection of any saline in order to demonstrate a negative aspiration to avoid injecting into blood vessels.

Then, the catheter was connected via an extension set (75 cm length, TUTA, Australia) to an infusion pump (IVAC Model P2000, UK) –See figure 3-8 containing one of the

following solutions in a 10 ml syringe (Becton Dickinson, Singapore): 5% sterile saline (hypertonic saline) or 0.9% sterile saline (isotonic saline).

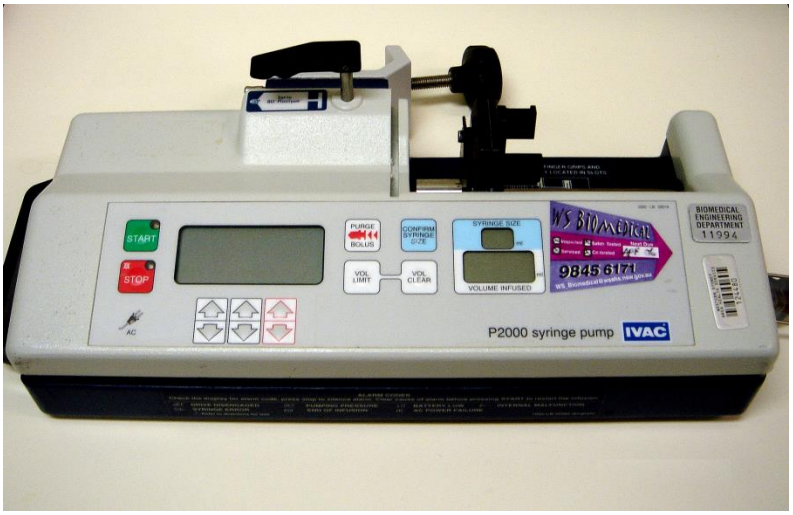


Figure 3-8: Infusion pump for the continuous infusion of the saline.

Participants were not aware of which solution would be infused first, although they could perceive which block was the painful one as soon as the infusion of hypertonic solution had started. The infusion of isotonic saline was used as a control for possible changes in jaw muscle activity and/or jaw forces due to volumetric changes within the muscle from the infusion of a solution and also was used alternately with the hypertonic saline infusion to minimize time-related effects.

This study aimed to induce moderate pain and therefore during the hypertonic saline infusion, pain intensity was maintained between 30-60 on a 100 mm visual analogue scale (VAS) (see figure 3-9 below) for approximately 10 minutes and this was achieved by continuous pump infusion.

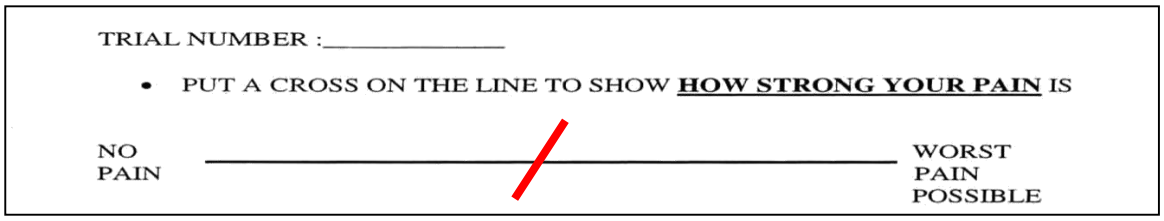


Figure 3-9: Pain intensity visual analogue scale (VAS). Each participant was instructed to mark on the line how intense the pain was at that time. The pain intensity was maintained at a moderate level by adjusting the infusion rate so that the participant made a mark somewhere between 30-60 mm on the 100 mm scale as shown on the figure.

The VAS is one of most popular methods used to assess pain intensity as it is reliable, and is validated with adequate sensitivity (Carlsson 1983). The VAS had anchors of “no pain” and “the worst pain possible”. A moderate level of pain (one mark around the middle of the 100 mm line) was maintained by noting the VAS score that was obtained after each task trial during blocks 2 and 3.

The VAS score is determined by measuring in millimetres from the left hand end of the line to the point that the patient marks. If the participant marked a score on a position that was visually equivalent to a value lower than 30/100 mm, the infusion rate of hypertonic saline was increased by 1-4 ml/hr. Similarly, after a VAS score was marked on a position equivalent to a value higher than 60/100 mm, the infusion rate was decreased or stopped temporarily until the VAS score marked after a subsequent trial returned to the 30-60 mm range. One of the operator’s sole task was to monitor and adjust the infusion rates.

The rates of infusion varied considerably between participants. For those participants who had hypertonic infusion as the first infusion block (i.e. block 2), the infusion rates

were determined according to their VAS scores as explained above, and the isotonic infusion rates used in block 3 followed the hypertonic parameters. For those participants who had isotonic saline as the first solution applied (block 2), the rate of isotonic saline infusion was set at 4 ml/h for the first 3 minutes and then increased to 6 ml/h for the remainder of the isotonic infusion block. The hypertonic rates of infusion for the subsequent block 3 were then adjusted according to the VAS scores as described above.

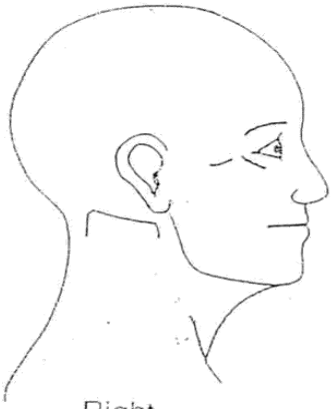
After the completion of each task trial (a single recording of a task) during both infusions (i.e. blocks 2 and 3), during both infusions (i.e. blocks 2 and 3), a recording was made of the pain intensity VAS on a paper version of the scale. In addition, each participant was asked to make a drawing of the maximum distribution of their pain on the front, back, right and left lateral-profile outline pictures of the head and neck. (see Figure 3-10 below).

TRIAL NUMBER :_____

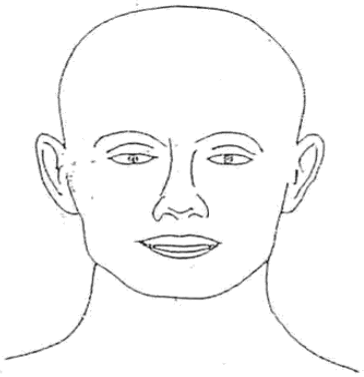
- PUT A CROSS ON THE LINE TO SHOW **HOW STRONG YOUR PAIN** IS

NO PAIN _____ WORST PAIN POSSIBLE

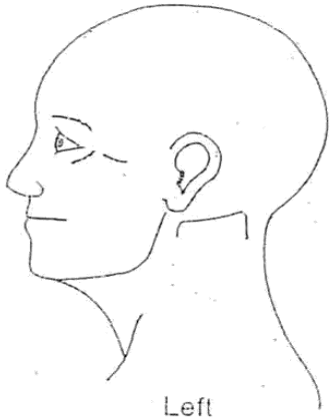
- DRAW THE AREA ON THE PICTURE TO SHOW **WHERE YOU FEEL PAIN**



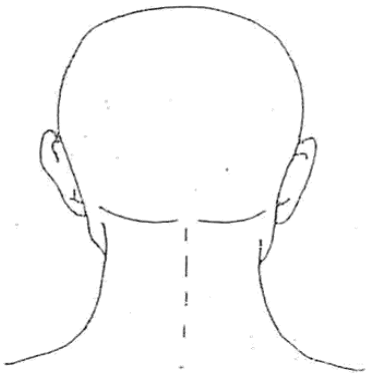
Right



Front



Left



Back

- 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 3-10 Visual analogue scale (VAS) and pain maps. This sheet was given to the participant to complete after each task trial throughout Blocks 2 and 3.

After each block of trials involving an infusion, each participant was asked to complete the McGill Pain Questionnaire (MPQ) (Melzack 1975) – see appendix 8.5.

The MPQ is a scale of pain rating that was developed at McGill University by Melzack and Torgerson (1971). It is a self-report questionnaire that helps individuals to give a description of the quality and intensity of the pain that they are experiencing. The MPQ is composed of 78 pain descriptors which fall into four categories, that is, sensory, affective, evaluative, and miscellaneous characteristics of a painful experience, and from which participants choose those that best describe their pain.

Three main types of data can be obtained from this questionnaire:

1- Pain rating index (PRI) – This consist of the total sum of the scale values of all the words chosen in a given category. In other words, the rank values of the words chosen by a patient are summed to obtain a separate score for each dimension. The maximum possible scores are: PRI sensory (42), PRI affective (14), PRI evaluative (5) and PRI miscellaneous (17). Higher scores indicate more severe pain.
2- The number of words chosen - (NWC) - is the sum score of the total number of descriptors that the participant chooses.
3- The present pain intensity - (PPI) – the number and word combination chosen indicates the overall intensity of pain (Melzack 1975).

The most frequent data to explain the results obtained from this questionnaire is the Pain Rating Index – PRI.

Figure 3-11 shows the front and lateral view of one participant after the placement of surface and intramuscular electrodes.

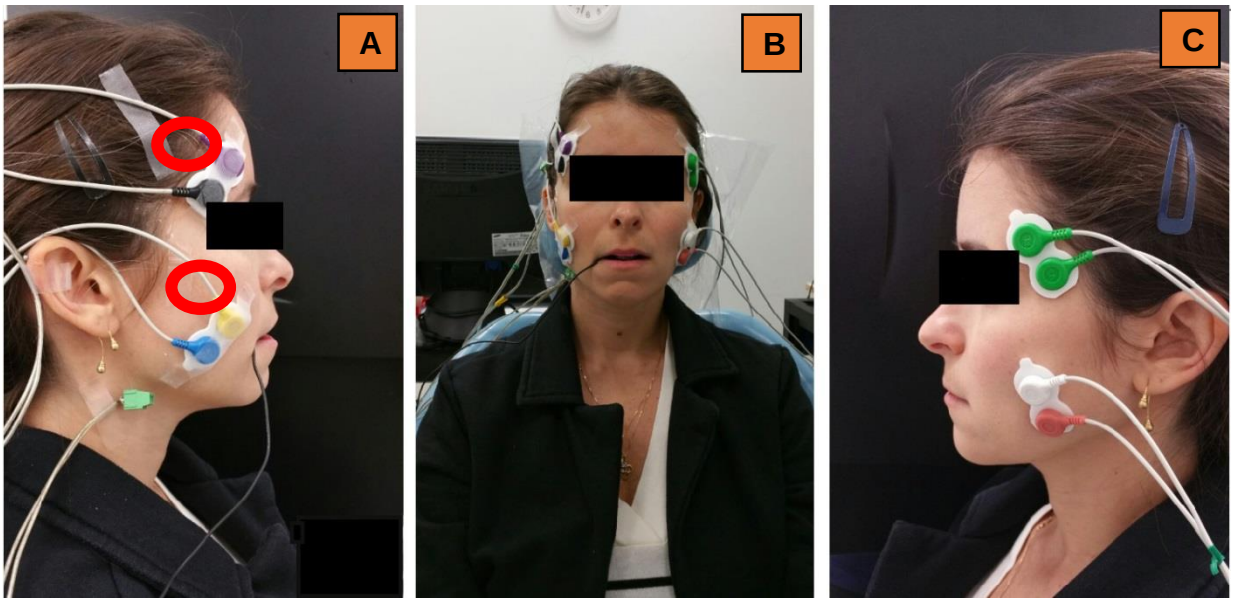


Figure 3-11: participant in front and lateral view. Panel A: right side of participant showing surface EMG for temporalis and masseter muscles, as well as the region of face where intramuscular electrodes were placed (circled in red). Panel B: front view of the participant with customised splints on the mouth and electrodes in place. Panel C: left side of participant showing surface EMG for temporalis and masseter muscle.

3.5 Data analysis

3.5.1 Participant demographic data/questionnaires

3.5.1.1 Participant demographic data

Participant demographic data was qualitatively described and tabulated with means and standard deviations.

3.5.1.2 RDC/TMD

The results of the RDC/TMD were reported qualitatively to confirm that the participant did not have TMD.

3.5.1.3 DASS 21 and PCS

Means and standard deviation scores were calculated for each scale of the DASS, and subscales of the PCS.

3.5.1.4 Infusions

The means and standard deviations of the total infused volume across the 20 participants during hypertonic saline and isotonic saline infusion were documented. A paired samples t-test was used to determine whether there was a significant difference in the volume of saline infused between the hypertonic and isotonic saline sessions across all participants.

3.5.1.5 VAS scores

The values for each repetition, means and standard deviations of the VAS scores across the 20 participants during hypertonic saline and isotonic saline infusion were documented.

Repeated measures Analysis of Variance (ANOVA) was done for the VAS scores of pain intensity to analyse possible effects of repeating the trial within an infusion block and, therefore to enable means of the repeated tasks within one block to be used in further analyses.

Paired sample t-tests were done to compare the following VAS pain intensity scores:

- Hypertonic block x isotonic block during slow ramp jaw closing task,
- Hypertonic block x isotonic block during fast ramp jaw closing task and,
- Hypertonic block x isotonic block during 2 step-levels jaw closing task.

3.5.1.6 Distribution of perceived pain

The distribution of perceived pain (location of pain during the hypertonic and isotonic saline infusion blocks) was also reported in tables.

3.5.1.7 McGill Pain questionnaire scores

The most cited word was calculated and described in terms of how often they appeared for the hypertonic saline and the isotonic saline infusion block.

Each pain rating index (PRI) was calculated for both infusion blocks (i.e. hypertonic saline infusion block and isotonic saline infusion block). For this analysis, the words that were not cited by any participant were excluded and the mean scales and mean scores were calculated for each of the pain descriptors from the MPQ based on how many times the word was cited and the scale and score given to that specific word (see Appendix 8.6).

Besides the score, each word was given a weight. To find the weight it was necessary to multiply the word score by a constant value established for each of the 20 different groups of words (Melzack 1975). After excluding the words that were not cited by any participant, a scale value and a weight value is established based on the remaining words and how many times each word is cited – See table 3-3.

This data were presented quantitatively to describe the computed value of the pain experience.

Scale value (scale x number of times cited)	**Weighted value (weight x number of times cited)**

Table 3-2: shows how the scale and weighted values were calculated

Paired T-tests were done to compare the scores of the mean scale and mean weight between the hypertonic saline infusion block and isotonic saline infusion block

3.5.2 Analysis of force

The force values of the script at which the participants performed the different tasks (slow ramp jaw closing task, fast ramp jaw closing task and 2 step-levels jaw closing tasks) were obtained. The force was recorded in volts and was then converted into Newtons (N), using the following mathematic formula:

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

Where the constant values are the gain of the force transducer channel (3 mV) and the rated capacity of the transducer (1142.857 mV/s).

The mean and standard deviation of the force values in Newtons (after being converted from volts) were calculated for each of the 3 trials of the jaw closing force tasks and graphs were generated of the force outputs. The graphs were used to identify the start and end points of the slow ramp jaw closing task, fast ramp jaw closing task and the most stable period of each step level for the 2 step-levels jaw closing task. For the ramp jaw closing tasks (fast and slow), the beginning of the increase in force was determined as the first deviation after a plateau or the initial rest period and the end

point was determined as the highest force level reached for each participant for that ramp task.

For the 2 step-levels jaw closing tasks, the most stable ~2 s period of force was chosen during each holding force level (Step 1 and Step 2). This was determined from the graph where the data indicated a consistently low standard deviation across the holding force period. Therefore, the most stable period with the lowest standard deviation was chosen for the analysis of the root mean square RMS of the EMG activity and single motor unit discrimination. Then, using the Spike2 program, two vertical cursors were created to define the boundary of the most stable period with the lowest standard deviation– See figure 3-12.

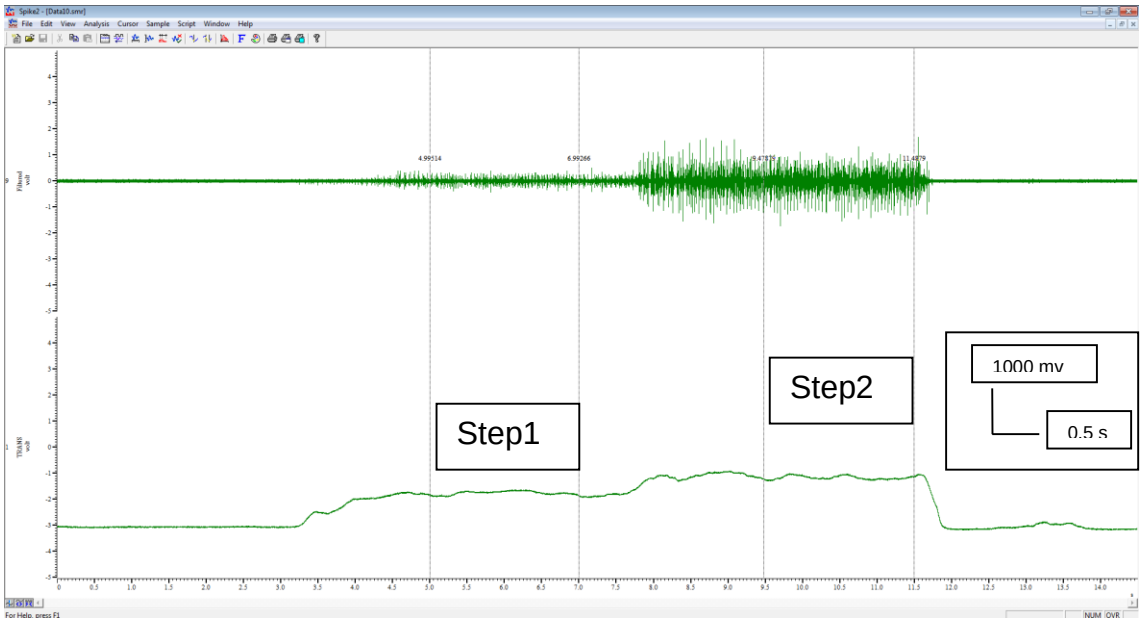


Figure 3-12: Screen shot taken from the EMG activity and the force output of one of the participants. Two vertical courses determining each of the stable periods for step 1 and step 2. X-axis: time in seconds (Sec). Y-axis: force transducer output in volts (V).

A repeated measures analysis of variance (ANOVA) was carried out to determine possible differences in the force values reached throughout the baseline block, hypertonic saline infusion block and isotonic saline infusion block namely:

- Force amplitude – the highest force achieved for each participant (N) – during each task repetition of the ramps tasks (3 slow ramp jaw closing tasks and 3 fast ramp jaw closing tasks);
- Force levels at step level 1 (N) during the first chosen time period of the 2 step-levels, during each task repetition of the 2 step-levels jaw closing task;
- Force levels step level 2 (N), during the second chosen time periods of the 2 step-levels, during each task repetition of the 2 step-levels jaw closing task.

Pairwise comparisons with Bonferroni corrections were done to investigate possible differences between the blocks, baseline, hypertonic saline infusion and isotonic saline infusion.

The force rates of the ramps jaw closing tasks were calculated by dividing the highest force the participant achieved by the number of seconds the participant took to achieve it. Those values were presented in tables. Paired t-tests calculated some possible significant difference between the following:

- Baseline slow ramp x hypertonic slow ramp
- Baseline slow ramp x isotonic slow ramp
- Hypertonic slow ramp x isotonic slow ramp
- Baseline fast ramp x hypertonic fast ramp

- Baseline fast ramp x isotonic fast ramp
- Hypertonic fast ramp x isotonic fast ramp

The rates between baselines (slow ramp x fast ramp), hypertonic infusions (slow ramp x fast ramp) and isotonic infusions (slow ramp x fast ramp) were also compared.

3.5.3 Root mean square (RMS) activity

In order to capture possible differences in the overall EMG activity for all of the participants, the data were analysed using the RMS of the EMG signal at each intramuscular electrode EMG site (right masseter and right temporalis muscle) for the 2 step-levels task.

The most stable period at each level of the 2 step-levels task was chosen as described above. The Spike 2 then limited the calculation of the RMS values to within the range between these 2 cursors (see Figure 3-12) and then, excel files were generated with RMS values for every trial and at each level of the 2 step-levels jaw closing task during the 4 blocks applied in this study.

A repeated-measures ANOVA was used for the 3 repetitions of each task for all the participants to determine whether there was a variance on the EMG activity between the repeated trials. If no significant effect for repeating was found, then the mean of the repetitions were calculated and used for further analysis of difference between the blocks.

3.5.4 Single motor unit (SMU) activity

From each trial of tasks from the baseline (block 1), hypertonic saline infusion, isotonic saline infusion and baseline 2 (block 2) blocks, SMU activity was discriminated in the right temporalis muscle.The present data analysis , did not include baseline 2 for the masseter EMG analysis only from the baseline, hypertonic saline infusion and isotonic saline infusion blocks, as the main aim of this study was to analyse the effects of pain on the non-painful muscle, in this case the temporalis.

For the slow ramp jaw closing task and the fast ramp jaw closing task, SMUs were discriminated through the whole force period until the point where a higher force would make it difficult to distinguish clearly the recruitment of additional SMUs. For each trial of the 2 step-levels jaw closing task, on the other hand, the 2 s of data with the lowest variability of force at each force level (as defined above, see Figure 3-12) were selected to be analysed.

Single motor units were discriminated based on SMU waveform using specialized software (Spike2, Cambridge Electronic Design, Cambridge, UK). This template matching software was used to identify initially SMUs within a recording. This software does allow some variation in the shape of the identified SMU and still will classify the unit as the same SMU. All SMUs that were classified by the algorithm as being the same SMU, were also manually confirmed that they were similar in amplitude and shape. For each trial of the tasks, an assessment was made of the occurrence or not of a SMU –See figure 3-13 and 3-14. The criteria for discriminating single motor units were:

1- Similarities in waveform;

2- A regular time of occurrence;

3- Firing within a continuous 2-s period.

As each kind of task had a minimum of 3 trials, in order to be considered present during one task, the SMU should fit all the mentioned criteria for at least 2 out of the 3 repeated trials.

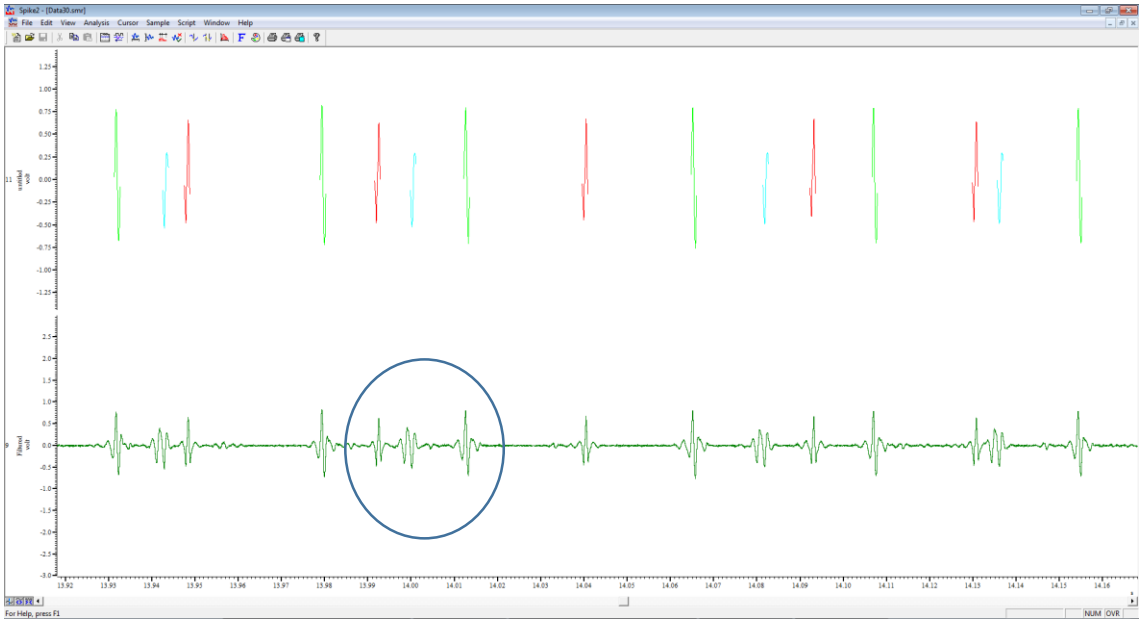


Figure 3-13: Spike2 screen showing three different waveforms (shapes) characterizing the discrimination of three different SMUs. On the bottom line it is possible to see the EMG signal and on the top line it is possible to see the discrimination of SMUs as identified by the waveform discrimination software (Spike2 Cambridge Electronic Design, Cambridge, UK); the software allocates different colours for each SMU. The 3 SMUs in the blue circle are enlarged in Figure 3-14.

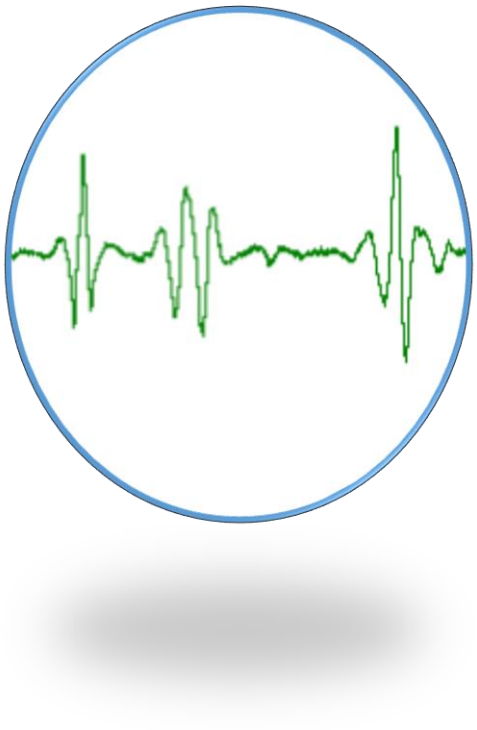


Figure 3-14: The three different SMUs discriminated according to the shape of the waveform and displayed on a zoom image.

Every SMU determined as present or not present during each jaw closing task in each block were tabulated for each muscle analysed (right temporalis and right masseter). This allowed an assessment of whether the particular SMU was active in each task and block or only in some tasks or blocks. For example, it was possible to determine whether an SMU was recruited (i.e. it was present) during a task in one or more blocks or whether an SMU was de-recruited (i.e. it was not present) during a task in one or more blocks.

An analysis was performed to compare the occurrence (if SMUs were recruited, de-recruited or presented no change) of SMUs during the hypertonic saline infusion block with their occurrences during the baseline, isotonic saline infusion and baseline 2 for the temporalis muscle, and with baseline and isotonic block for the masseter muscle.

One of the functions of the baseline was to allow the participant to become accustomed to the experimental tasks, as well as a comparator for the infusion blocks. However, the comparison between the isotonic saline and hypertonic saline blocks was the principal comparison used in this study for defining whether there was recruitment, de-recruitment or no change in SMU EMG activity. This is because the only differences between the isotonic saline and the hypertonic saline infusion blocks was the presence of pain and that one was done after the other, that is, there was an order-related factor to consider. The order factor was however, addressed by alternating the sequence of type of infusion between participants.

An analysis was also performed to determine whether the changes in the pattern of occurrence of SMUs at the temporalis and masseter muscle sites were consistent with the proposals of the Vicious Cycle Theory or the Pain Adaptation Model. If the SMU was recruited during the hypertonic saline infusion session but was inactive (i.e. not present) in the isotonic session, then the pattern of occurrence of the SMU was considered to be consistent the Vicious Cycle Theory as the pain, according to this theory, would cause “muscle hyperactivity” and one manifestation of this “hyperactivity” would be SMU recruitment. On the other hand, if the SMU becomes inactive (i.e. not present) during the hypertonic infusion session in comparison with the isotonic session, this pattern of occurrence was considered to be consistent with the Pain Adaptation Model where pain is associated with decreased agonist muscle activity and one manifestation of this would be de-recruitment of one or more SMUs. The net result of this pain effect as proposed by the Pain Adaptation Model would be slower and smaller movements to prevent further injury and help healing.

For each trial of the 2 step-levels jaw closing task, the firing rates of each identified SMU at each level of force were calculated as the number of times the SMU fired divided by the time. The firing frequencies of each of the SMUs were qualitatively compared between the hypertonic and isotonic block of recording sessions. A repeated measures analysis of variance (ANOVA) was carried out to determine a significant effect of pain on the muscle activity and multiple comparisons with Bonferroni corrections investigated if the hypertonic saline infusion block (pain) had significantly different firing rates to that of the baseline and isotonic saline block recordings.

For each trial of each of the slow and fast ramp jaw closing tasks, calculation of the threshold for onset of firing in relation to force (N) for each SMU was done. The time point that in each trial the SMU commenced firing continuously for at least one second was defined as the threshold value. A repeated measures analysis of variance (ANOVA) was carried out to determine a significant effect of pain on the SMU threshold values and multiple comparisons with Bonferroni corrections investigated if the hypertonic saline infusion block (pain) threshold values were significantly different to the SMU thresholds for the baseline and isotonic saline block recordings.

The sequence of recruitment of SMUs from the right temporalis muscle was further analysed to check whether the sequence of recruitment would be altered according to the infusion or not. For this purpose, only units that were present in the baseline, hypertonic and isotonic blocks were considered and participants that only had one unit that was present in the three blocks were also not considered in this analysis as it is

not possible to provide a recruitment sequence. The sequence of recruitment on which each motor unit appeared within a task in one block, was tabulated and compared with other blocks.

3.5.5 Psychological values

A quantitative analysis was done to determine a possible correlation between a change in SMU characteristics (occurrences) and psychological variables. The PCS and DASS-21 scores of the individuals where the occurrence of SMU activity did not alter during any of the infusions for the temporalis muscle was compared with the same scores of those participants where the occurrence of SMU activity did change in at least one block of infusion and those values were presented in tables.

4 RESULTS:

The Results chapter is divided into 6 sections: 4.1. demographics, questionnaires and infusion data, 4.2 force amplitude, rates, levels, 4.3 root mean square (RMS) analysis, 4.4. Single motor unit (SMU) analysis, 4.5. Masseter and temporalis occurrence and consistency with VCT and PAM – comparison and 4.6. Psychological variable influence on jaw muscle pain.

4.1 Demographics, questionnaires and infusion data:

4.1.1 Age, gender and solution injected first:

20 participants were recruited - 15 females (75%) and 5 males (25%) - aged between 22-40 yrs (mean (SD) 29.5 (4.3) yrs). Table 4-1 lists sex and age and the sequence of infusion. All the participants were able to perform all the different tasks (slow ramp jaw closing task, fast ramp jaw closing task, 2 step-levels jaw closing task) under the 4 different blocks (baseline, hypertonic saline infusion, isotonic saline infusion, baseline 2).

Participant ID	Sex	Age (yrs)	Solution applied first
1	F	26	Hypertonic
2	F	30	Isotonic
3	M	33	Hypertonic
4	F	29	Isotonic
5	F	29	Hypertonic
6	F	40	Isotonic
7	M	25	Hypertonic
8	F	28	Isotonic
9	M	29	Hypertonic
10	F	39	Isotonic
11	F	28	Hypertonic
12	F	31	Isotonic
13	M	27	Hypertonic
14	M	31	Isotonic
15	F	22	Hypertonic
16	F	24	Hypertonic
17	F	30	Isotonic
18	F	31	Hypertonic
19	F	29	Isotonic
20	F	28	Hypertonic
Mean		29.5	
SD		4.3	

Table 4-1: Participants' ID, sex, age, and solution injected first. SD: Standard deviation

4.1.2 Scores from the RDC/TMD history and clinical examination

Of the 20 participants, only one reported to have felt pain in the past month, although the pain was described as a one-time occurrence, and none of the participants reported pain in any part of their body prior to starting on the day of the experiment.

Five participants reported to have heard a pop or a clicking while chewing, and 11 participants reported grinding or clenching their teeth while sleeping at night, while only 3 reported grinding or clenching their teeth during the day.

Among all the participants, none reported any jaw problem that would interfere with any of the following activities: chewing, drinking, exercising, eating hard foods, eating soft foods, smiling/laughing, sexual activity, cleaning teeth or face, yawning, swallowing, talking, and having their usual facial appearance.

During the clinical examination, 7 participants presented with joint sounds during jaw opening or closing or both. And finally, when the examiner palpated different areas of the participants' muscles on the face, head and neck, only 1 participant indicated mild pain on intraoral palpation of the lateral pterygoid area.

4.1.3 Scores from the DASS-21 questionnaire

The scores for depression, anxiety and stress, and means and standard deviations (SD) for each participant are shown in Table 4-2 below. The table also shows that within the 3 scales scores, stress had the highest mean (1.5) followed by anxiety (0.5) and depression (0.2).

Participant	Stress	Depression	Anxiety
1	0	0	0
2	0	3	1
3	3	0	2
4	0	0	0
5	0	0	0
6	2	1	0
7	0	0	0
8	0	0	0
9	1	0	0
10	1	0	0
11	0	0	0
12	1	0	0
13	2	0	1
14	3	0	1
15	3	0	1
16	0	0	0
17	2	0	0
18	7	1	3
19	5	0	1
20	0	0	0
Mean	1.5	0.2	0.5
SD	1.9	0.7	0.8

Table 4-2: DASS-21 scores (sum of the relevant statements) for depression, anxiety and stress for each participant along with mean and standard deviation (SD) for each of the scale scores.

4.1.4 Scores from the PCS questionnaire:

Pain catastrophising scores for each participant are shown in Table 4-3. This table shows the total score and the 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 23 out of a possible score of 52. The table also shows that the mean total score was 8.35 and that within the 3 subscales, rumination and helplessness had the highest group mean (3.25) for both followed by magnification (1.85).

Participant	Rumination	Magnification	Helplessness	Total Score
1	2	2	0	4
2	1	0	1	2
3	3	0	0	3
4	11	3	9	23
5	1	1	0	2
6	2	2	2	6
7	3	4	6	13
8	0	1	1	2
9	0	0	0	0
10	4	3	6	13
11	8	2	4	14
12	0	0	0	0
13	1	0	1	2
14	0	0	1	1
15	2	0	2	4
16	10	5	7	22
17	2	2	3	7
18	6	5	10	21
19	4	3	7	14
20	5	4	5	14
Mean	3.2	1.8	3.2	8.3
SD	3.3	1.7	3.2	7.7

Table 4-3: PCS scores for rumination, magnification and helplessness subscales and total PCS scores along with their respective means and standard deviations (SD).

4.1.5 Infusions

A standardized infusion paradigm with a bolus of 0.1-0.3 ml of 5% hypertonic saline was infused for all the 20 participants. The infusion rate during continuous infusion was controlled between 0.6-17 ml/h, with differences in rates between participants being adjusted in order to maintain pain intensity ideally at a constant level of a 30-60 mm on a 100-mm VAS.

The total amount of infused solution (*i.e.,* initial bolus volume plus continuous infusion volume) for each participant is shown in Tables 4-4 and 4-5. There was a high variability in the VAS scores between individuals (see next section) and there was a very high infusion volume required for some participants (e.g. 1.9 ml, participant 9), and a very low volume (e.g. 0.1 ml, participant 6) was required for others.

The means and standard deviations of the total infused volume across the 20 participants during the hypertonic saline and isotonic saline infusions were 0.8 ml (SD: 0.5 ml) and 0.7 ml (SD: 0.4 ml), respectively. A paired samples t-test found no significant difference (p=0.74) between the total volume infused for hypertonic (0.8 ml) and isotonic saline solutions (0.7 ml).

Participant	Volume of hypertonic saline infused and infusion rate		
	Bolus infusion (ml)	Total volume (ml)	Rate (ml/h)
1	0.1	0.2	2
2	0.1	0.4	No data
3	0.2	0.3	2-4
4	0.2	0.5	2-4
5	0.2	0.9	4-10
6	0.1	0.1	1
7	0.2	1.3	4-10
8	0.3	1.5	4-12
9	0.2	1.9	9-14
10	0.2	0.9	9
11	0.2	0.7	0.6
12	0.2	0.6	3
13	0.2	1.9	10 -17
14	0.1	0.6	4
15	0.2	1.4	4-15
16	0.3	0.8	5-13
17	0.2	1	4-9
18	0.3	1	4-8
19	0.2	0.4	4-1
20	0.2	0.2	1-2
MEAN	0.2	0.8	
SD	0.06	0.5	

Table 4-4: Initial bolus infusion volume (ml), and total volume of infused hypertonic saline (5%) (ml) and rate of infusion (ml/h) obtained from each participant. No data: data was not recorded.

Participant	Volume of isotonic saline infused and infusion rate		
	Bolus infusion (ml)	Total volume (ml)	Rate (ml/h)
1	0.1	0.4	2
2	0.2	0.3	No data
3	0.2	0.3	2-4
4	0.2	0.2	1-2
5	0.2	1	4-9
6	0.2	0.4	4
7	0.2	1.3	4-11
8	0.2	0.7	4
9	0.2	1.3	5-9
10	0.2	0.6	4
11	0.2	0.6	4
12	0.2	0.7	4
13	0.2	1.5	10-14
14	0.2	0.6	4
15	0.2	1.4	4-14
16	0.3	0.8	5-13
17	0.2	0.6	3-4
18	0.3	1	4-8
19	0.2	0.4	4-1
20	0.2	0.2	1-2
MEAN	0.2	0.7	
SD	0.03	0.4	

Table 4-5: Initial bolus infusion volume (ml), and total volume of infused isotonic saline (0.9%) (ml) and rate of infusion (ml/h) obtained from each participant. No data: data was not recorded

4.1.6 Scores from the VAS:

The VAS scores are illustrated in Tables 4-6, 4-7, 4-8, 4-9, 4-10, and 4-11 below. Most of the participants reported moderate pain for the hypertonic saline block and minimal pain intensity or no pain at all for all the tasks under isotonic block of infusion. However, 5 participants reported to have felt pain during the isotonic saline block of infusion and their scores varied from 3.3/100 to 34.7/100 during the slow ramp jaw closing task (Table 4-7), 4 participants felt pain with their scores varying from 10/100 to 35/100 during the fast ramp jaw closing task (Table 4-9), and 4 participants scored from 10/100 to 32.7/100 on the VAS of the 2 step-levels jaw closing task (Table 4-11) during the isotonic saline block of infusion. For the hypertonic block, the highest mean VAS score was recorded from Participant 20 during the slow ramp jaw closing task at approximately 71/100 whereas the lowest mean score was noted by Participant 9 also during the slow ramp jaw closing tasks at 20/100.

Repeated measures Analysis of Variance (ANOVA) of the VAS pain intensity scores after each trial of a jaw closing task during the hypertonic saline infusion showed no significant effect of repeating the trial (3 trials of slow ramp jaw closing task, 3 trials of fast ramp jaw closing task, 3 trials of 2 step-levels jaw closing task) with $p=0.308$ for the slow ramp jaw closing task, $p=0.107$ for the fast ramp jaw closing task, and $p=0.379$ for the 2 step-levels jaw closing task. During the isotonic saline infusion there were also no significant effects for repetitions with $p=0.45$ for the slow ramp jaw closing task, $p=0.362$ for the fast ramp jaw closing task, $p=0.248$ for the 2 step-levels jaw closing task.

Because there was no significant difference found in the repetitions, the means of each block were used in a paired samples t-test of within participants and revealed that the VAS scores obtained during hypertonic saline infusion were significantly greater ($p<0.001$) than the VAS scores obtained during the isotonic saline infusion for each of the three different tasks (slow ramp jaw closing task, fast ramp jaw closing task, 2 step-levels jaw closing tasks) – See figures 4-1, 4-2, 4-3.

- **SLOW RAMP JAW CLOSING TASK:**

	Hypertonic saline infusion				
Participant	SR1	SR2	SR3	Mean	SD
1	50	50	60	53.3	5.7
2	50	40	42	44	5.3
3	47	55	55	52.3	4.6
4	23	43	24	30	11.3
5	30	46	56	44	13.1
6	57	60	54	57	3
7	50	30	30	36.7	11.5
8	40	40	40	40	0
9	20	20	20	20	0
10	23	15	20	19.3	4
11	50	40	40	43.3	5.8
12	43	37	47	42.3	5
13	53	25	20	32.7	17.8
14	50	50	50	50	0
15	45	47	43	45	2
16	39	24	20	27.7	10
17	54	39	40	44.3	8.4
18	41	57	49	49	8
19	60	60	50	56.7	5.7
20	75	75	65	71.7	5.7
Mean	**45**	**42.6**	**41.2**	**43**	**1.9**
SD	**13.5**	**15.1**	**14.4**	**12.8**	**0.8**

Table 4-6: VAS (visual analogue scale) scores (mean and SD in millimetres) for all participants, during slow ramp jaw closing task across hypertonic saline infusion block. SD: standard deviation. SR 1: Slow ramp jaw closing task first repetition. SR 2: Slow ramp jaw closing task second repetition. SR 3: Slow ramp jaw closing task third repetition.

	Isotonic saline infusion				
Participant	SR1	SR2	SR3	Mean	SD
1	0	0	0	0	0
2	35	34	35	34.7	0.6
3	0	0	0	0	0
4	21	14	25	20	5.6
5	0	0	0	0	0
6	8	11	7	8.7	2.1
7	0	0	0	0	0
8	0	0	0	0	0
9	0	0	0	0	0
10	0	0	0	0	0
11	0	0	0	0	0
12	10	0	0	3.3	5.8
13	0	0	0	0	0
14	0	0	0	0	0
15	0	0	0	0	0
16	0	0	0	0	0
17	0	0	0	0	0
18	0	0	0	0	0
19	10	10	10	10	0
20	0	0	0	0	0
Mean	**4.2**	**3.4**	**3.8**	**3.9**	**0.4**
SD	**9.1**	**8.4**	**9.5**	**8.9**	**0.5**

Table 4-7: VAS (visual analogue scale) scores (mean and SD in millimetres) for all participants, during slow ramp jaw closing task across isotonic saline infusion block. SD: standard deviation. SR 1: Slow ramp jaw closing task first repetition. SR 2: Slow ramp jaw closing task second repetition. SR 3: Slow ramp jaw closing task third repetition.

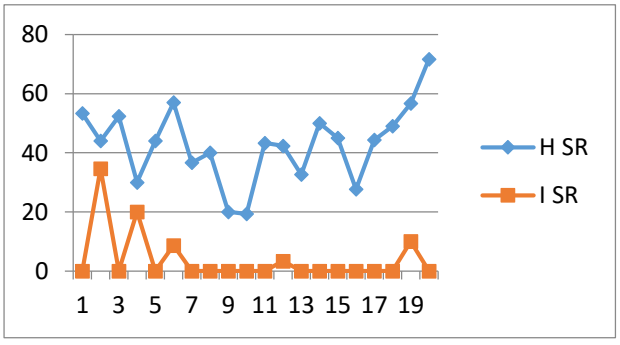


Figure 4-1: Mean VAS scores (y axis) from each participant (x axis) during slow ramp jaw closing task during hypertonic saline infusion block (blue) and during isotonic saline infusion block (orange). H SR: Slow ramp jaw closing task during hypertonic saline infusion. I SR: Slow ramp jaw closing task during isotonic saline infusion.

- **FAST RAMP JAW CLOSING TASK:**

	Hypertonic saline infusion				
Participant	FR1	FR2	FR3	Mean	SD
1	50	51	52	51	1
2	42	45	43	43.3	1.5
3	51	54	49	51.3	2.5
4	35	41	32	36	4.6
5	40	23	37	33.3	9.1
6	31	29	16	25.3	8.1
7	40	40	50	43.3	5.8
8	40	30	30	33.3	5.8
9	40	50	50	46.7	5.8
10	11	30	30	23.7	11
11	50	60	60	56.7	5.8
12	49	50	53	50.7	2.1
13	31	35	40	35.3	4.5
14	50	50	50	50	0
15	43	47	49	46.3	3.1
16	18	50	65	44.3	24
17	40	40	37	39	1.7
18	35	35	45	38.3	5.8
19	50	50	60	60.7	5.8
20	60	62	60	60.7	1.1
Mean	**40.3**	**43.6**	**45.4**	**43.5**	**2.6**
SD	**11.6**	**10.7**	**12.4**	**10.5**	**0.8**

Table 4-8: VAS (visual analogue scale) scores (mean and SD in millimetres) for all participants, during fast ramp jaw closing task across hypertonic saline infusion block. SD: standard deviation. FR 1: Fast ramp jaw closing task first repetition. FR 2: Fast ramp jaw closing task second repetition. FR 3: Fast ramp jaw closing task third repetition.

	Isotonic saline infusion				
Participant	FR1	FR2	FR3	Mean	SD
1	0	0	0	0	0
2	35	33	37	35	2
3	0	0	0	0	0
4	30	40	20	30	10
5	0	0	0	0	0
6	20	12	7	13	6.7
7	0	0	0	0	0
8	0	0	0	0	0
9	0	0	0	0	0
10	0	0	0	0	0
11	0	0	0	0	0
12	0	0	0	0	0
13	0	0	0	0	0
14	0	0	0	0	0
15	0	0	0	0	0
16	0	0	0	0	0
17	0	0	0	0	0
18	0	0	0	0	0
19	10	10	10	10	0
20	0	0	0	0	0
Mean	**4.7**	**4.7**	**3.7**	**4.4**	**0.6**
SD	**10.7**	**11.4**	**9.3**	**10.3**	**1.1**

Table 4-9: VAS (visual analogue scale) scores (mean and SD in millimetres) for all participants, during fast ramp jaw closing task across isotonic saline infusion block. SD: standard deviation. FR 1: Fast ramp jaw closing task first repetition. FR 2: Fast ramp jaw closing task second repetition. FR 3: Fast ramp jaw closing task third repetition.

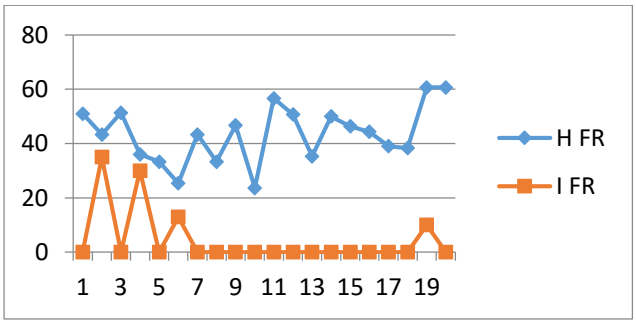


Figure 4-2: Mean VAS scores (y axis) from each participant (x axis) during fast ramp jaw closing task during hypertonic saline infusion block (blue) and isotonic saline infusion block (orange). H FR: Fast ramp jaw closing task during hypertonic saline infusion. I FR: Fast ramp jaw closing task during isotonic saline infusion.

- **2 STEP-LEVELS JAW CLOSING TASK:**

	Hypertonic saline infusion				
Participant	ST 1	ST 2	ST 3	Mean	SD
1	53	49	49	50.3	2.3
2	45	40	44	43	2.6
3	45	45	44	44.7	0.6
4	43	36	33	37.3	5.1
5	34	44	49	42.3	7.6
6	52	50	50	50.7	1.1
7	40	50	50	46.7	5.8
8	30	40	40	36.7	5.8
9	60	60	50	56.7	5.8
10	40	50	50	46.7	5.8
11	50	50	40	46.7	5.8
12	51	56	53	53.3	2.5
13	45	43	46	44.7	1.5
14	50	50	50	50	0
15	25	35	37	32.3	6.4
16	47	56	50	51	4.6
17	49	50	56	51.7	3.8
18	45	40	50	45	5
19	60	60	60	60	0
20	61	60	50	57	6.1
Mean	**46.2**	**48.2**	**47.5**	**47.3**	**1**
SD	**9.4**	**7.7**	**6.4**	**7**	**1.5**

Table 4-10: VAS (visual analogue scale) scores (mean and SD in millimetres) for all participants, during 2 step-levels jaw closing task across hypertonic saline infusion block. SD: standard deviation. ST 1: 2 step-levels jaw closing task first repetition. ST 2: 2 step-levels jaw closing task second repetition. ST 3: 2 step-levels jaw closing task third repetition.

	Isotonic saline infusion				
Participant	ST 1	ST 2	ST 3	Mean	SD
1	0	0	0	0	0
2	35	33	30	32.7	2.5
3	0	0	0	0	0
4	24	11	16	17	6.6
5	0	0	0	0	0
6	21	21	20	20.7	0.6
7	0	0	0	0	0
8	0	0	0	0	0
9	0	0	0	0	0
10	0	0	0	0	0
11	0	0	0	0	0
12	0	0	0	0	0
13	0	0	0	0	0
14	0	0	0	0	0
15	0	0	0	0	0
16	0	0	0	0	0
17	0	0	0	0	0
18	0	0	0	0	0
19	10	10	10	10	0
20	0	0	0	0	0
Mean	**4.5**	**3.7**	**3.8**	**4**	**0.4**
SD	**10.1**	**8.8**	**8.5**	**9**	**0.9**

Table 4-11: VAS (visual analogue scale) scores (mean and SD in millimetres) for all participants, during 2 step-levels jaw closing task across isotonic saline infusion block. SD: standard deviation. ST 1: 2 step-levels jaw closing task first repetition. ST 2: 2 step-levels jaw closing task second repetition. ST 3: 2 step-levels jaw closing task third repetition.

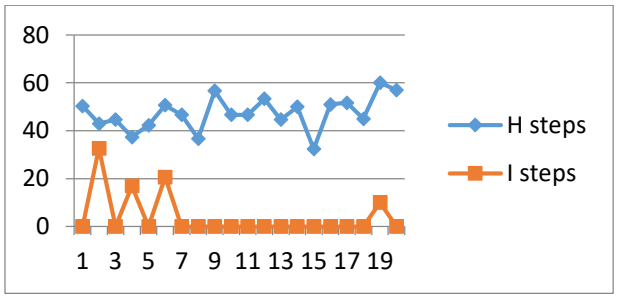


Figure 4-3: Mean VAS scores (y axis) from each participant (x axis) during 2 step-levels jaw closing task during hypertonic saline infusion block (blue) and isotonic saline infusion block (orange). H SR: 2 step-levels jaw closing task during hypertonic saline infusion. I SR: 2 step-levels jaw closing task during isotonic saline infusion.

4.1.7 Distribution of perceived pain

The evoked pain was visually mapped after the performance of every trial of each task during both saline infusion blocks. A summary of the pain maps from all participants is shown in Table 4-12.

This table indicates that for the hypertonic block of infusion, all participants (n=20) described localised pain in the area of the right masseter that was infused with the hypertonic saline solution. Participants 2 and 17 also reported referred pain in the right anterior temporalis muscle. For the isotonic block of infusion, on the other hand, participants 2, 4, 6 and 19 reported localised pain in the area of the right masseter that was infused with isotonic saline solution and participant 2 also reported referred pain in the right anterior temporalis. – See figure 4-4.

Hypertonic					Isotonic saline infusion			
Participant	Right Masseter	Right Temporalis	Other	Pain free after	Participant	Right Masseter	Right Temporalis	Other
1	Yes	No	No	5 min	1	No	No	No
2	Yes	Yes	No	7 min	2	Yes	Yes	No
3	Yes	No	No	3 min	3	No	No	No
4	Yes	No	No	5 min	4	Yes	No	No
5	Yes	No	No	4 min	5	No	No	No
6	Yes	No	No	8 min	6	Yes	No	No
7	Yes	No	No	9 min	7	No	No	No
8	Yes	No	No	1 min	8	No	No	No
9	Yes	No	No	3 min	9	No	No	No
10	Yes	No	No	5 min	10	No	No	No
11	Yes	No	No	2 min	11	No	No	No
12	Yes	No	No	5 min	12	No	No	No
13	Yes	No	No	2 min	13	No	No	No
14	Yes	No	No	6 min	14	No	No	No
15	Yes	No	No	4 min	15	No	No	No
16	Yes	No	No	1 min	16	No	No	No
17	Yes	Yes	No	2 min	17	No	No	No
18	Yes	No	No	5 min	18	No	No	No
19	Yes	No	No	6 min	19	Yes	No	No
20	Yes	No	No	6 min	20	No	No	No

Table 4-12: Location of pain during the hypertonic and isotonic saline infusion blocks. For hypertonic and isotonic saline infusion blocks, Yes: pain map located over the muscle (right masseter or right temporalis). No: pain map not located over the muscle (right masseter, right temporalis or other muscle). Highlighted Participant 2 and 17 who reported referred pain on the right temporalis muscle during the hypertonic saline infusion block, and Participant 2, who have reported pain on the right masseter and right temporalis muscle during the isotonic saline infusion block and Participant 4, 6 and 19 who have reported pain in the right masseter during the isotonic saline infusion block.

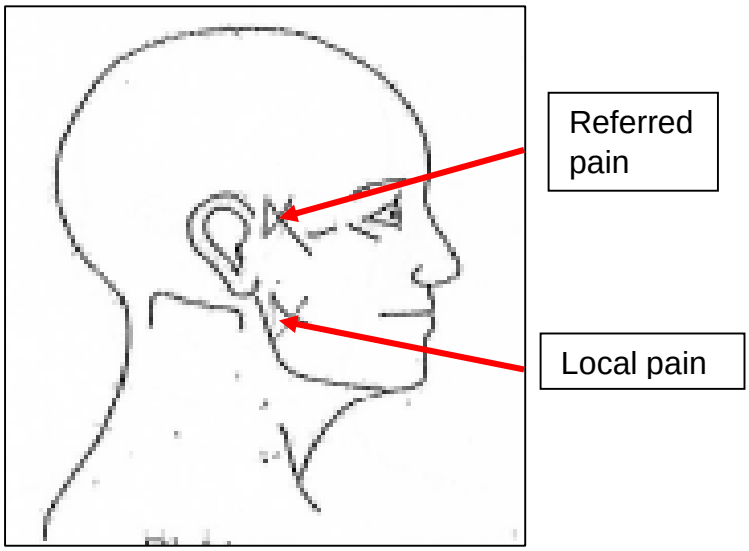


Figure 4-4: Example of one participant where pain was felt not only at the site of the saline infusion (masseter muscle, local pain) but also in the region of the temporalis muscle (referred pain).

In summary, during the hypertonic saline infusion block all 20 participants (100%) felt pain in the masseter muscle and of these, 2 participants also felt pain in the region of the temporalis muscle.

In the isotonic saline infusion block, some participants marked a pain area for the right masseter only or right masseter and right temporalis. Of the 20 participants, 4 participants (Participant 2, 4, 6, 19) felt pain in the masseter muscle and only 1 (Participant 2) felt pain in the region of the temporalis muscle. There was no report of pain felt elsewhere on the face.

4.1.8 Scores from the McGill questionnaire

The most cited word in this study for the hypertonic block of infusion was “annoying” (14/20), followed by “aching” (13/20), “pressing” (12/20) and “jumping” (8/20). For the most cited words during the isotonic block of infusion, “boring” was cited by 3 people and “annoying” by 2.

The following tables (Table 4-13 and 4-14) detail the words chosen for both hypertonic and isotonic blocks of infusion:

Descriptors	Group	Words	Number of times the word was cited in Hypertonic saline	scale	weight	scale value (scale x number of times cited)	weighted value (weight x number of times cited)
Sensory	1	Pulsing	1	3	2.07	3	2.07
		Beating	2	5	3.45	10	6.9
	2	Jumping	8	1	1.38	8	11.04
		Shooting	5	3	4.14	15	20.7
	3	Pricking	3	1	0.93	3	2.79
		Boring	6	2	1.86	12	11.16
		Drilling	3	3	2.79	9	8.37
		Stabbing	2	4	3.72	8	7.44
	4	Sharp	5	1	1.59	5	7.95
		Cutting	2	2	3.18	4	6.36
	5	Pinching	4	1	0.81	4	3.24
		Pressing	12	2	1.62	24	19.44
		Gnawing	1	3	2.43	3	2.43
		Cramping	1	4	3.24	4	3.24
		Crushing	1	5	4.05	5	4.05
	6	Pulling	1	2	2.38	2	2.38
	7	Hot	2	1	1.28	2	2.56
		Burning	1	2	2.56	2	2.56
		Searing	1	4	5.12	4	5.12
	8	Smarting	1	3	2.1	3	2.1
		Stinging	3	4	2.8	12	8.4
	9	Dull	1	1	0.72	1	0.72
		Sore	5	2	1.44	10	7.2

Descriptors	Group	Words	Number of times the word was cited in Hypertonic saline	scale	weight	scale value (scale x number of times cited)	weighted value (weight x number of times cited)
Sensory	9	Hurting	4	3	2.16	12	8.64
		Aching	13	4	2.88	52	37.44
		Heavy	1	5	3.6	5	3.6
	10	Taut	6	2	1.88	12	11.28
		Splitting	1	4	3.76	4	3.76
Affective	11	Tiring	3	1	1.74	3	5.22
		Exhausting	3	2	3.48	6	10.44
	12	Sickening	2	1	2.22	2	4.44
	13	Terrifying	1	3	5.61	3	5.61
	14	Punishing	1	1	1.32	1	1.32
		Grueling	1	2	2.64	2	2.64
Evaluative	16	Annoying	14	1	1.01	14	14.14
		Miserable	3	3	3.03	9	9.09
		Intense	3	4	4.04	12	12.12
Miscellaneous	17	Radiating	2	2	2.44	4	4.88
		Penetrating	6	3	3.66	18	21.96
	18	Tight	2	1	0.81	2	1.62
		Numb	1	2	1.32	2	1.32
	19	Cool	1	1	1	1	1
		Freezing	1	3	3	3	3
	20	Nagging	1	1	1.15	1	1.15
		Dreadful	5	4	4.6	20	23
		Total chosen	146				

Table 4-13: Table with words from the McGill questionnaire endorsed during the hypertonic saline infusion block. The table lists the number of times the word was cited, the scale, and weight, and the scale value and weighted values that were calculated.

Descriptors	Group	Words	Number of times the word was cited in Isotonic saline	scale	Weight	scale value (scale x number of times cited)	weighted value (weight x number of times cited)
Sensory	1	Beating	1	5	3.45	5	3.45
	2	Shooting	1	3	4.14	3	4.14
	3	Boring	3	2	1.86	6	5.58
		Drilling	1	3	2.79	3	2.79
	4	Sharp	1	1	1.59	1	1.59
	5	Pressing	1	2	1.62	2	1.62
		Gnawing	1	3	2.43	3	2.43
	6	Pulling	1	2	2.38	2	2.38
	7	Burning	1	2	2.56	2	2.56
	8	Stinging	1	4	2.8	4	2.8
	9	Sore	1	2	1.44	2	1.44
		Hurting	1	3	2.16	3	2.16
		Aching	1	4	2.88	4	2.88
		Heavy	1	5	3.6	5	3.6
	10	Tender	1	1	0.94	1	0.94
Affective	11	Tiring	1	1	1.74	1	1.74
	13	Fearful	1	1	1.87	1	1.87
Evaluative	16	Annoying	2	1	1.01	2	2.02
		Troublesome	1	2	2.02	2	2.02
Miscellaneous		Penetrating	1	3	3.66	3	3.66
		Total chosen	23				

Table 4-14: Table with words from the McGill questionnaire endorsed during isotonic saline infusion block. The table lists the number of times the word was cited, the scale, and weight, and the scale value and weighted values that were calculated.

For each descriptor, a pain rating index (PRI) was calculated for the 2 different blocks of infusions and each is shown in the following tables (Tables 4-15, 4-16).

Hypertonic saline infusion:

Pain rating index	Sum scale	Sum weight	Mean scale	Mean weight
Sensory	238	212.9	8.5	7.6
Affective	17	29.7	2.8	4.9
Evaluative	35	35.3	11.8	11.8
Miscellaneous	51	57.9	6.4	7.2
Total (mean)	85.2	84	7.4	7.9

Table 4-15: Total sum scale, total sum weight and pain rating index (PRI) descriptor class scores to describe the pain according to the obtained scale and weighted scores after the hypertonic saline infusion block.

Isotonic saline infusion block:

Pain rating index	Sum scale	Sum weight	Mean scale	Mean weight
Sensory	46	40.4	3.1	2.7
Affective	2	3.6	1	1.8
Evaluative	4	4	2	2
Miscellaneous	3	3.7	3	3.7
Total (mean)	13.7	12.9	2.3	2.5

Table 4-16: Total sum scale, total sum weight and pain rating index (PRI) descriptor class scores to describe the pain according to the obtained scale and weighted scores after the isotonic saline infusion block.

Paired T-tests were done to compare the scores of the mean scale and mean weight between the hypertonic saline infusion block and isotonic saline infusion block and found a significant ($p<0.005$) difference in the each of the PRIs analysed.

4.2 Force amplitude, rates, levels

Force values, captured in volts were mathematically converted to Newtons with the aid of the Excel program. As stated in the Methods, the highest force achieved for each of the slow and fast ramp jaw closing tasks were compared between each repetition within each participant. A similar calculation was done for the most stable 2-second period for each of step 1 and step 2, in the 2 step-levels jaw closing task. The value found on the excel sheet was confirmed visually on the force output from the spike2 program (See figures 4-5, 4-6 and 4-7 below).

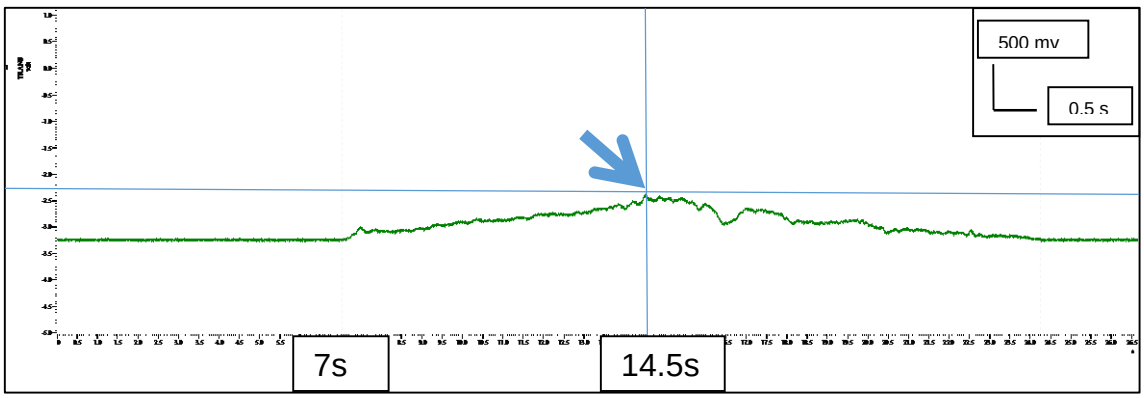


Figure 4-5: Example of force output for a slow ramp jaw closing task. An arrow is placed indicating the exact moment where this particular participant achieved the highest force. It can be seen that the time from the lowest to the highest level of force took about 7.5 s (from 7.0 s to 14.5 s). X-axis: time in seconds (Sec). Y-axis: force transducer output in volts (V).

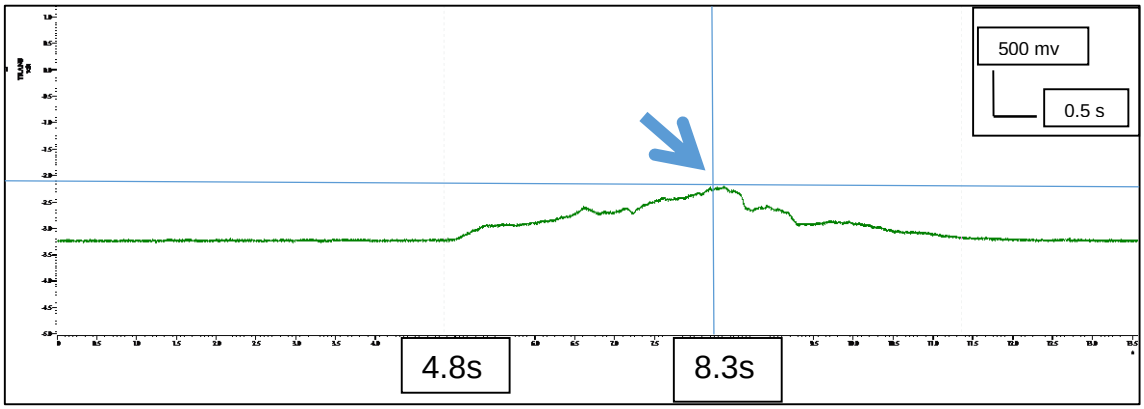


Figure 4-6: Example of force output for a fast ramp jaw closing task. An arrow is placed indicating the exact moment where this particular participant achieved the highest force. The time from the lowest force level to the highest one took about 3.5 s approximately (from 4.8 s to 8.3 s). X-axis: time in seconds (Sec). Y-axis: force transducer output in volts (V).

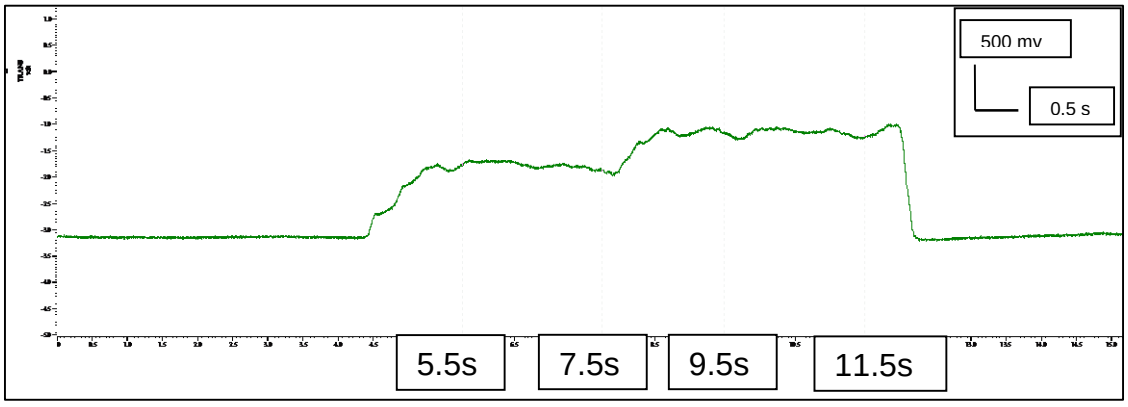


Figure 4-7: Example of force output for a 2 step-levels jaw closing task with the most stable period of approximately 2 seconds for each step level defined. See Methods for criterion used to define the most stable 2-s period. X-axis: time in seconds (Sec). Y-axis: force transducer output in volts (V).

Force rates were also analysed based on the same Excel program, by dividing the value in newtons of the highest point of the exerted force for the number of seconds that the participant took to exert that force.

4.2.1 Force amplitude

Tables 4-17 and 4-18 show the mean of the highest force values in newtons across the 3 repetitions of each task of the jaw closing ramp tasks (slow ramp jaw closing task and fast ramp jaw closing task).

- **SLOW RAMP JAW CLOSING TASK:**

ID	Baseline		Hypertonic Saline		Isotonic Saline	
	Mean	SD	Mean	SD	Mean	SD
1	95.0	1.9	83.5	2.5	83.9	4.4
2	33.4	5.0	41.5	4.3	36.7	1.0
3	51.2	9.7	37.1	5.1	38.5	6.4
4	56.3	9.0	50.6	4.3	53.9	9.5
5	59.5	5.3	58.1	2.0	57.0	1.3
6	52.9	4.6	51.2	4.7	54.8	1.6
7	37.0	3.8	40.6	2.8	43.1	2.5
8	51.3	5.4	55.3	3.3	56.0	1.7
9	47.5	3.8	52.7	12.0	62.9	7.5
10	62.1	1.4	56.0	2.2	62.6	3.8
11	51.1	1.9	59.3	6.7	58.7	3.1
12	55.6	2.3	50.7	12.2	54.6	0.8
13	59.9	3.3	61.2	2.2	61.8	2.7
14	65.3	2.6	62.2	3.2	48.3	5.0
15	58.5	6.0	59.7	2.2	56.8	3.4
16	55.9	0.6	52.2	1.7	46.3	0.2
17	66.3	4.5	60.2	2.5	54.3	7.9
18	49.0	6.0	33.8	15.3	53.7	1.9
19	39.9	19.4	42.2	8.5	55.6	4.4
20	49.0	5.9	42.5	9.7	57.6	2.5

Table 4-17: Mean of the highest force in N for the slow ramp jaw closing tasks during baseline, hypertonic saline infusion block and isotonic saline infusion block. ID: participant number. SD: standard deviation. For comparisons between the hypertonic saline infusion block and the isotonic saline infusion block, the highlighted values show the block where the force was smaller than the other block. .

- **FAST RAMP JAW CLOSING TASK:**

ID	Baseline		Hypertonic Saline		Isotonic Saline	
	Mean	SD	Mean	SD	Mean	SD
1	89.6	4.1	89.8	10.2	69.8	12.9
2	126.9	5.3	128.2	5.1	107.2	6.7
3	49.1	10.8	53.8	2.2	46.5	1.9
4	67.1	1.8	66.1	1.4	60.2	8.4
5	59.3	2.2	61.3	2.2	65.9	2.0
6	51.9	1.6	54.5	6.0	53.8	5.7
7	43.8	1.8	50.0	6.5	44.0	1.8
8	52.5	3.4	66.5	13.8	62.1	2.5
9	51.9	1.3	62.4	12.1	69.1	4.7
10	64.3	5.2	65.9	8.0	75.1	3.0
11	43.5	4.8	55.0	2.6	46.6	4.1
12	36.0	0.3	36.1	5.3	34.9	0.8
13	56.5	4.4	51.9	3.1	60.1	5.6
14	49.9	4.5	64.8	4.7	70.9	7.0
15	58.4	5.3	61.0	0.9	60.1	2.4
16	54.7	4.4	60.8	3.6	53.0	2.2
17	67.5	2.9	61.2	2.3	58.8	7.7
18	51.8	4.8	47.0	4.2	55.4	3.9
19	64.3	1.2	64.4	1.7	63.7	2.5
20	55.1	3.8	51.2	1.1	61.3	2.8

Table 4-18: Mean of the highest force in N for the fast ramp jaw closing tasks during baseline, hypertonic saline infusion block and isotonic saline infusion block. ID: participant number. SD: standard deviation. For comparisons between the hypertonic saline infusion block and the isotonic saline infusion block, the highlighted values show the block where the force was smaller than the other block. .

Qualitatively, it was possible to notice that, for the slow ramp jaw closing task and for comparison among the 3 blocks, 7 participants (participant 2, 7, 8, 9, 11, 13, 19) exerted the lowest force during the baseline block, 8 participants (participant 1, 3, 4, 6, 10, 12, 18, 20) exerted the lowest force during the hypertonic saline infusion block and lastly, 5 participants (participant 5, 14, 15, 16, 17) exerted the lowest force during the isotonic saline infusion block.

For the fast ramp jaw closing task and for comparison among the 3 blocks, it was possible to notice that 9 participants (participant 5, 6, 7, 8, 9, 10, 11, 14, 15) exerted the lowest during the baseline block, only 3 participants (participant 13, 18, 20) exerted the lowest force during the hypertonic saline infusion block and lastly, 8 participants (participant 1, 2, 3, 4, 12, 17, 18, 19) exerted the lowest force during the isotonic saline infusion block.

A repeated measures analysis of variance (ANOVA) determined that there were no significant effects ($p>0.05$) of the repetitions on the force values for each participant during each task trial of the jaw closing ramp tasks (slow ramp jaw closing task and fast ramp jaw closing task).

- For the repetitions during the slow ramp jaw closing tasks:
    - $p=0.41$ for the baseline block;
    - $p=0.06$ for the hypertonic saline infusion block;
    - $p=0.63$ for the isotonic saline infusion block.

Because no effect of repetition during the slow ramp jaw closing tasks was found, further analysis (pairwise comparisons with Bonferroni corrections) were done using the mean of the repetitions and found that there was no significant effect of the block ($p=0.33$) on the force values during the slow ramp jaw closing task.

- For the repetitions during the fast ramp jaw closing tasks:

- p=0.44 for the baseline block;
- p=0.22 for the hypertonic saline infusion block;
- p=0.94 for the isotonic saline infusion block.

Because no effect of repetition during the fast ramp jaw closing tasks was found, further analysis (pairwise comparisons with Bonferroni corrections) were done using the mean of repetitions and found that there was no significant effect of the block (p=0.32) on the force values during the fast ramp jaw closing task.

Therefore, force values were not affected by the pain induction and all the participants were able to perform all the trials of the jaw closing ramp tasks (slow ramp jaw closing task and fast ramp jaw closing task) for the three blocks analysed in this study.

4.2.2 Force rates

Tables 4-19 and 4-20 show the force rate values (N/s) for the slow ramp jaw closing task and fast ramp jaw closing task.

- **SLOW RAMP JAW CLOSING TASK:**

Baseline	Hypertonic	Isotonic

8.9	8.2	8.4
5.2	7.0	6.7
8.0	3.2	2.7
3.3	3.0	3.0
3.1	3.3	3.2
3.3	3.2	3.1
2.9	2.7	2.7
3.0	3.4	3.5
3.3	3.6	3.5
3.7	3.4	4.2
2.8	3.5	3.3
3.5	3.3	3.5
3.1	3.8	3.5
3.7	3.4	2.7
3.2	3.3	3.4
3.2	3.0	2.7
3.5	3.0	2.8
2.9	2.3	2.8
3.7	3.2	3.4
3.1	2.6	3.2

Table 4-19: Force rate values for slow ramp jaw closing task

Paired t-tests showed no significant difference between baseline and hypertonic ($p=0.4$), baseline and isotonic ($p=0.4$) or hypertonic and isotonic ($p=0.9$) for the slow ramp jaw closing task.

- **FAST RAMP JAW CLOSING TASK:**

Baseline	Hypertonic	Isotonic
18.8	18.7	18.2
21.1	22.6	12.3
16.4	8.5	7.0
8.0	8.7	6.9
6.9	8.0	7.6
6.0	7.2	5.9
7.1	7.9	7.0
7.6	9.1	9.6
7.6	8.5	9.2
9.0	9.4	10.1
5.4	6.7	6.2
5.3	5.8	5.4
8.1	6.4	8.3
5.8	7.0	8.3
7.5	8.4	7.0
9.4	9.4	7.9
7.8	7.7	7.2
6.5	6.2	6.6
8.8	8.4	7.5
7.2	7.0	8.2

Table 4-20: Force rate values for fast ramp jaw closing task

Paired t-tests showed no significant difference between baseline and hypertonic ($p=0.9$), baseline and isotonic ($p=0.3$) or hypertonic and isotonic ($p=0.2$) for the fast ramp jaw closing task. However, comparing the rates between slow ramp and fast ramp, paired t tests showed a significant difference between baseline slow ramp and baseline fast ramp, hypertonic slow ramp and isotonic fast ramp, isotonic slow ramp and isotonic fast ramp ($p<0.005$) for the three conditions.

4.2.3 Force levels

Tables 4-21 and 4-22 show the mean of the force values in newtons across the 3 repetitions at each level of the 2 step-levels jaw closing task (step 1 and step 2).

- **STEP 1 OF THE 2 STEP-LEVELS JAW CLOSING TASK:**

ID	Baseline		Hypertonic		Isotonic	
	Mean	SD	Mean	SD	Mean	SD
1	18.0	3.4	16.7	2.4	18.8	2.7
2	68.5	2.3	65.7	1.6	62.2	5.8
3	50.3	2.7	49.7	2.5	47.3	3.1
4	49.8	2.0	52.4	1.6	47.0	3.0
5	27.3	2.0	29.9	2.5	27.8	1.6
6	45.8	5.3	52.3	0.7	52.4	5.4
7	48.2	4.3	44.8	2.9	45.0	6.6
8	27.0	4.4	25.0	0.9	29.0	1.8
9	23.8	1.1	18.6	1.3	15.2	4.1
10	12.0	2.8	8.5	1.1	12.0	1.5
11	23.2	5.7	19.8	6.8	18.1	2.2
12	33.1	0.9	32.0	2.3	31.8	0.8
13	29.7	1.2	29.6	4.1	27.0	1.2
14	40.7	4.4	34.2	3.5	44.4	3.4
15	26.3	6.5	30.7	0.5	28.5	1.8
16	23.0	3.8	18.5	1.6	14.3	0.9
17	34.8	1.2	32.5	5.3	36.1	2.6
18	33.4	2.0	33.1	0.3	34.9	2.0
19	11.2	0.7	10.4	1.9	10.9	0.3
20	39.7	3.5	41.0	0.5	39.8	3.0

Table 4-21: Mean of the highest force in N for the step 1 of the 2 step-levels jaw closing tasks during baseline, hypertonic saline infusion block and isotonic saline infusion block. ID: participant number. SD: standard deviation. For comparisons between the hypertonic saline infusion block and the isotonic saline infusion block, the highlighted values show the block where the force was smaller than the other block.

- **STEP 2 OF THE 2 STEP-LEVELS JAW CLOSING TASK:**

ID	Baseline		Hypertonic		Isotonic	
	Mean	SD	Mean	SD	Mean	SD
1	36.2	0.1	41.2	1.4	40.3	1.8
2	113.7	3.3	118.5	0.6	115.8	5.9
3	66.3	0.8	65.1	1.4	64.0	0.6
4	67.2	2.7	70.6	1.1	68.5	1.3
5	61.3	4.3	61.8	3.2	62.9	2.0
6	88.9	6.0	95.9	3.7	99.1	5.0
7	74.0	0.4	67.1	4.0	67.4	4.0
8	60.1	0.0	63.3	2.7	59.9	2.0
9	47.8	2.7	42.2	1.9	37.9	3.9
10	51.0	0.3	51.7	4.1	48.2	1.4
11	67.6	0.7	59.5	7.2	61.5	3.1
12	47.1	1.1	47.1	0.6	47.0	2.8
13	65.2	3.0	62.4	1.5	61.9	0.5
14	148.2	3.6	141.8	3.3	153.0	3.6
15	50.5	7.0	54.9	0.7	51.4	1.7
16	56.6	3.5	51.9	0.9	50.9	1.9
17	66.1	2.2	59.5	2.7	64.7	1.5
18	51.6	2.1	51.9	1.1	52.5	0.6
19	31.2	4.5	30.7	3.7	30.2	2.1
20	72.2	4.4	75.2	4.0	72.2	1.8

Table 4-22: Mean of the highest force in N for the step 2 of the 2 step-levels jaw closing tasks during baseline, hypertonic saline infusion block and isotonic saline infusion block. ID: participant number. SD: standard deviation. For comparisons between the hypertonic saline infusion block and the isotonic saline infusion block, the highlighted values show the block where the force was smaller than the other block.

For the step 1 of the 2 step-levels jaw closing task and for comparison among the 3 blocks, it was possible to notice that 4 participants (participant 5, 6, 15, 20) exerted the lowest force during the baseline block, 8 participants (participant 1, 7, 8, 10, 14, 17, 18, 19) exerted the lowest force during the hypertonic saline infusion block and lastly, 8 participants (participant 2, 3, 4, 9, 11, 12, 13, 16) exerted the lowest force during the isotonic saline infusion block.

And lastly, for the step 2 of the 2 step-levels jaw closing task and for comparison among the 3 blocks, it was possible to notice that 7 participants (participant 1, 2, 4, 5, 6, 15, 18) exerted the lowest force during the baseline block, 4 participants (participant 7, 11, 14, 17) exerted the lowest force during the hypertonic saline infusion block and lastly, 9 participants (participant 3, 8, 9, 10, 12, 13, 16, 19, 20) exerted the lowest force during the isotonic saline infusion block.

A repeated measures analysis of variance (ANOVA) determined that there was no significant effect ($p > 0.05$) of the repetitions on the force values for each participant during each task trial of the 2 step-levels jaw closing task.

- For the repetitions during the step 1 of the 2 step-levels jaw closing tasks:
  - $p=0.45$ for the baseline block;
  - $p=0.58$ for the hypertonic saline infusion block;
  - $p=0.69$ for the isotonic saline infusion block.

Because no effect of repetition during the step 1 of the 2 step-levels jaw closing tasks was found, further analysis were done using the mean of the repetitions and found no significant effect of the block ($p=0.3$) on the force level during the step 1 of the 2 step-levels jaw closing task.

- For the repetitions during the step 2 of the 2 step-levels jaw closing tasks:
  - $p=0.94$ for the baseline block;

- p=0.48 for the hypertonic saline infusion block;
- p=0.27 for the isotonic saline infusion block.

Because no effect of repetition during the step 2 of the 2 step-levels jaw closing tasks was found, further analysis were done using the mean of repetitions and found no significant effect of the block (p=0.76) on the force level during the step 2 of the 2 step-levels jaw closing task.

Therefore, force values were not affected by the pain induction and all the participants were able to perform all the trials of the 2 step-levels jaw closing tasks for the three blocks analysed in this study. Paired T-tests was also done to calculate if step 2 was significantly greater than step 1 under the three conditions analysed and it was found a significant difference for the three comparisons:

- p=0.002 for baseline step 1 x baseline step 2;
- p=0.003 for hypertonic step 1 x hypertonic step 2;
- p=0.000 for isotonic step 1 x isotonic step 2.

4.3 Root mean square (RMS)

In order to capture possible differences in the EMG activity of the temporalis and masseter muscles between the hypertonic and isotonic saline blocks of infusion, two measures were utilized: Root Mean Square (RMS) activity from the intramuscular EMG

recordings for the 2 steps-levels jaw closing tasks and a single motor unit analysis for slow and fast ramps jaw closing tasks and the 2 step-levels jaw closing tasks.

Among the 3 trials done for each task in each block of infusion, a repeated-measures ANOVA was done to determine the existence of any effect of repeating the jaw tasks on the RMS values of the EMG activity in each trial.

Tables 4-23 and 4-24 show the results of the repetition analysis for the masseter and temporalis muscle EMG activities, respectively.

	Masseter step 1	Masseter step 2
Baseline	P= 0.6	P= 0.39
Hypertonic saline	P= 0.36	P= 0.91
Isotonic saline	P= 0.67	P= 0.42
Baseline 2	P= 0.53	P= 0.27

Table 4-23: For the intramuscular activity of the right masseter muscle, no significant effect of repetitions was found for step 1 during baseline (p=0.6), hypertonic saline infusion (p=0.36), isotonic saline infusion (p=0.67), and baseline 2 (p=0.53); and for step 2 during baseline (p=0.39), hypertonic saline infusion (p=0.91), isotonic saline infusion (p=0.42), and baseline 2 (p=0.27).

	Temporalis step 1	Temporalis step 2
Baseline	P= 0.45	P= 0.11
Hypertonic	P= 0.33	P= 0.42
Isotonic	P= 0.71	P= 0.3
Baseline 2	P= 0.37	P= 0.42

Table 4-24: For the intramuscular activity of right temporalis, no significant effect of repetitions was found for step 1 during baseline (p=0.45), hypertonic saline infusion (p=0.33), isotonic saline infusion (p=0.71), and baseline 2 (p=0.37); and for step 2 during baseline (p= 0.11), hypertonic saline infusion (p=0.42), isotonic saline infusion (p=0.3), and baseline 2 (p=0.42).

Because there was no significant effect ($p > 0.05$) of repeating the tasks on the activity of the right masseter or the activity of the right temporalis muscles from the intramuscular electrodes, it was possible to use the mean EMG activity of the repetitions for further analysis. Pairwise comparisons were used to determine the presence of an overall significant effect of the pain block (hypertonic saline infusion) compared to the other blocks. This was done for each muscle, at each of the step levels from the 2 step-levels jaw closing task. There was no significant ($p>0.05$) effect of the pain (hypertonic saline infusion block) found for the right masseter step 1 ($p=0.4$), right masseter step 2 ($p=0.36$), right temporalis step 1 ($p= 0.21$), or right temporalis step 2 ($p= 0.16$).

Tables 4-25 and 4-26 show the RMS values of each muscle for each step level. Note that, although no effect of pain was significantly different, variability was found between participants.

Right masseter intramuscular electrode:

ID	right masseter intra step 1				right masseter intra step 2			
	Base	Hyper	Iso	Base 2	Base	Hyper	Iso	Base 2
1	0.17	0.34	0.26	0.16	0.21	0.19	0.20	0.20
2	0.08	0.28	0.19	8.69	4.44	6.57	5.50	5.50
3	0.80	0.89	1.34	1.15	1.25	1.20	1.22	1.22
4	1.35	0.97	1.24	1.04	1.14	1.09	1.12	1.12
5	0.36	0.80	0.74	0.62	0.68	0.65	0.67	0.67
6	0.90	1.00	0.92	0.65	0.79	0.72	0.75	0.75
7	1.52	1.38	2.00	1.93	1.97	1.95	1.96	1.96
8	2.07	0.83	1.48	1.06	1.27	1.16	1.22	1.22
9	0.32	0.69	0.66	1.12	0.89	1.01	0.95	0.95
10	3.40	3.80	3.95	3.04	3.50	3.27	3.38	3.38
11	0.48	0.57	0.59	0.24	0.41	0.33	0.37	0.37
12	0.60	0.58	0.70	1.06	0.88	0.97	0.93	0.93
13	0.84	0.13	0.25	0.31	0.28	0.30	0.29	0.29
14	0.53	0.29	0.65	0.40	0.53	0.46	0.50	0.50
15	0.30	0.32	0.32	0.27	0.29	0.28	0.29	0.29
16	0.66	0.23	0.16	0.31	0.23	0.27	0.25	0.25
17	0.68	1.29	1.08	1.20	1.14	1.17	1.15	1.15
18	0.44	0.23	0.20	0.22	0.21	0.21	0.21	0.21
19	0.30	0.31	0.36	0.30	0.33	0.32	0.32	0.32
20	0.45	0.44	0.30	0.15	0.23	0.19	0.21	0.21

Table 4-25: Means of the repetitions for the RMS intramuscular EMG activity for the right masseter for both step 1 and step 2 of the 2 step-levels jaw closing task and for the four blocks of tasks. ID: number of participant. Base = baseline block. Hyper = hypertonic block of infusion. Iso = Isotonic block of infusion. Base 2= Baseline 2. Highlighted: block with the increased RMS activity when a comparison is made between isotonic and hypertonic saline infusion blocks.

Right temporalis intramuscular electrode:

ID	right temporalis intra step 1				right temporalis intra step 2			
	Base	**Hyper**	**Iso**	**Base 2**	**Base**	**Hyper**	**Iso**	**Base 2**
1	0.77	0.81	0.83	0.63	0.73	0.68	0.70	0.55
2	1.11	0.94	1.16	0.90	1.03	0.96	1.00	0.94
3	1.19	4.57	1.93	2.28	2.10	2.19	2.15	2.24
4	0.83	0.76	1.03	0.86	0.95	0.91	0.93	1.39
5	0.26	0.45	0.42	0.71	0.57	0.64	0.60	1.21
6	3.71	4.30	3.92	4.15	4.04	4.09	4.06	3.14
7	0.62	1.11	1.03	0.37	0.70	0.54	0.62	0.19
8	0.17	0.11	0.11	0.12	0.11	0.12	0.12	0.13
9	0.87	0.17	0.22	0.23	0.22	0.23	0.22	0.23
10	0.78	0.47	0.55	0.40	0.47	0.44	0.46	0.31
11	1.67	1.67	1.89	1.31	1.60	1.46	1.53	0.35
12	1.15	1.58	1.37	0.68	1.02	0.85	0.94	0.42
13	0.61	0.48	0.73	0.62	0.68	0.65	0.67	0.75
14	0.11	0.12	0.14	0.12	0.13	0.13	0.13	0.07
15	0.30	0.42	0.39	0.33	0.36	0.34	0.35	0.21
16	0.35	0.30	0.39	0.36	0.38	0.37	0.37	0.18
17	0.72	1.22	0.91	0.94	0.93	0.94	0.93	0.14
18	0.56	0.69	0.46	0.51	0.49	0.50	0.49	0.49
19	0.15	0.18	0.21	0.17	0.19	0.18	0.18	0.08
20	0.06	0.06	0.07	0.05	0.06	0.06	0.06	0.05

Table 4-26: Means of the repetitions for the RMS intramuscular EMG activity for the right temporalis for both step 1 and step 2 of the 2 step-levels jaw closing task and for the four blocks of tasks. ID: number of participant. Base = baseline block. Hyper = hypertonic block of infusion. Iso = Isotonic block of infusion. Base 2= Baseline 2. Highlighted: increased RMS activity in comparison between isotonic and hypertonic saline.

For the right masseter during step 1 of the 2 step-levels jaw closing task, 10 participants (participant 1, 2, 5, 6, 9, 15, 16, 17, 18, 20) showed an increase in RMS activity which was consistent with VCT and 10 participants (participant 3, 4, 7, 8, 10, 11, 12, 13, 14, 19) showed a decrease in RMS activity which was consistent with the PAM during hypertonic saline infusion in comparison with isotonic saline infusion. For the same comparison, at the right masseter during step 2 of the 2 step-levels jaw

closing task, 7 participants (participant 1, 2, 9, 12, 13, 16, 17) showed an increase in RMS activity which was consistent with VCT and 13 participants (participant 3, 4, 5, 6, 7, 8, 10, 11, 14, 15, 18, 19, 20) showed a decrease in RMS activity which was consistent with the PAM.

For the right temporalis, during step 1 of the 2 step-levels jaw closing task, 10 participants (participant 3, 5, 6, 7, 8, 12, 15, 17, 18, 20) showed an increase in RMS activity which was consistent with VCT and 10 participants (participant 1, 2, 4, 9, 10, 11, 13, 14, 16, 19) showed a decrease in RMS activity which was consistent with the PAM during hypertonic saline infusion in comparison with isotonic saline infusion. For the same comparison, at the right temporalis during step 2 of the 2 step-levels jaw closing task, 9 participants (participant 3, 5, 6, 9, 16, 17, 18, 19, 20) showed an increase in RMS activity which was consistent with VCT and 11 participants (participant 1, 2, 4, 7, 8, 10, 11, 12, 13, 14, 15) showed a decrease in RMS activity which was consistent with the PAM.

The overall means of the RMS EMG activity from the intramuscular recording from the right masseter muscle and right temporalis muscle across all participants during each step level during each recording session (baseline, hypertonic, isotonic, baseline 2) are shown in Figures 4-8 and 4-9.

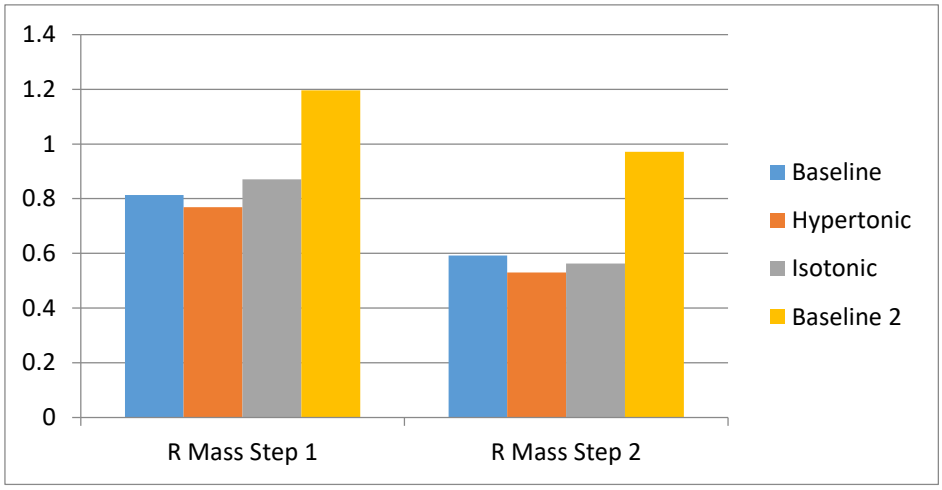


Figure 4-8 The overall means of the root mean square (RMS) EMG activity from the right masseter (R Mass) across all participants during each step level (Step 1 and Step 2) during each recording session (baseline, hypertonic saline, isotonic saline, Baseline 2).

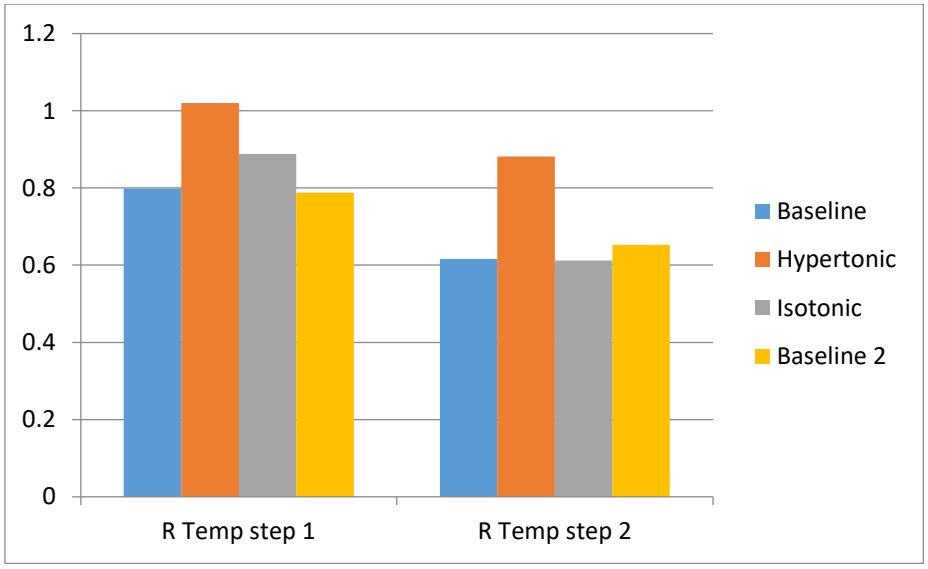


Figure 4-9: The overall means of the root mean square (RMS) EMG activity from the right temporalis (R Temp) across all participants during each step level (Step 1 and Step 2) during each recording session (baseline, hypertonic, isotonic, Baseline 2).

Interestingly, although no significant effect of the pain (hypertonic saline infusion block) between blocks, the means of the repetitions and the standard error were higher in the baseline 2 for the right masseter muscle step 1 and step 2, and higher for the

hypertonic block for the right temporalis muscle step 1 and step 2 as shown in the following tables (See in Tables 4-27, 4-28, 4-29, 4-30).

RIGHT MASSETER STEP 1:

Estimates

Measure: MEASURE_1

blocks	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
1 Baseline	.813	.174	.449	1.178
2 Hypertonic	.769	.179	.394	1.144
3 Isotonic	.871	.198	.458	1.285
4 Baseline 2	**1.196**	**.425**	.306	2.086

Table 4-27: The overall means of the root mean square (RMS) EMG activity from the right masseter across all participants during step 1.

RIGHT MASSETER STEP 2:

Estimates

Measure: MEASURE_1

blocks	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
1 Baseline	.592	.109	.364	.821
2 Hypertonic	.530	.086	.350	.710
3 Isotonic	.563	.111	.331	.796
4 Baseline 2	**.972**	**.422**	.089	1.855

Table 4-28: The overall means of the root mean square (RMS) EMG activity from the right masseter across all participants during step 2.

RIGHT TEMPORALIS STEP 1:

Estimates

Measure: MEASURE_1

blocks	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
1 Baseline	.799	.180	.422	1.176
2 Hypertonic	**1.020**	**.282**	.430	1.610
3 Isotonic	.888	.202	.466	1.310
4 Baseline 2	.788	.210	.348	1.229

Table 4-29: The overall means of the root mean square (RMS) EMG activity from the right temporalis across all participants during step 1.

RIGHT TEMPORALIS STEP 2:

Estimates

Measure: MEASURE_1

blocks	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
1 Baseline	.616	.098	.410	.822
2 Hypertonic	**.882**	**.235**	.391	1.373
3 Isotonic	.612	.144	.311	.912
4 Baseline 2	.653	.180	.276	1.030

Table 4-30: The overall means of the root mean square (RMS) EMG activity from the right temporalis across all participants during step 2.

4.4 Single motor unit analyses:

4.4.1 Muscles from which intramuscular recordings were made:

Single motor units were recorded from two muscles in this study: right masseter and right temporalis. Table 4-31 shows the muscles from which intramuscular EMG recordings were able to be obtained from each participant. Single motor unit activity was able to be recorded from the temporalis muscle in 16 participants and from the masseter muscle in 17 participants. In the remaining participants, it was not possible to record an EMG signal from which SMUs could be discriminated in one or both muscles. This was because of technical issues that arose during the recording.

ID	Masseter	Temporalis
1	Yes	Yes
2	No	Yes
3	Yes	No
4	No	Yes
5	Yes	Yes
6	Yes	Yes
7	Yes	Yes
8	Yes	Lost
9	Yes	Lost
10	Yes	Yes
11	Yes	Yes
12	Yes	Yes
13	Yes	Yes
14	Yes	Yes
15	No	Yes
16	Yes	Yes
17	Yes	Yes
18	Yes	Yes
19	Yes	Yes
20	Yes	No
Total:	17	16

Table 4-31: Table of successful masseter and temporalis muscle intramuscular recordings, that is, whether good quality EMG activity (i.e. activity that allowed single motor unit discrimination) was recorded from the muscle or not. ID: number of the participant. No: No/poor quality EMG activity. Yes: EMG activity allowed single motor unit discrimination. Lost: signal was lost in the middle of the experiment so those EMG data for those participants were not considered.

From the data of the 16 participants from which SMU recordings were obtained from the temporalis muscle, 83 SMUs were discriminated and they were classified in numeric order. From the data of the 17 participants from which SMU recordings were obtained from the masseter muscle, 58 SMUs were discriminated and they were also classified in numeric order.

4.4.2 Occurrence of single motor unit for right temporalis and right masseter muscle

The main question of this study was whether the pain-induced reorganization of activity that has been previously demonstrated within one jaw muscle (right masseter) also occurs in other jaw muscles (right temporalis).

Therefore, 83 units were discriminated from the temporalis muscle in one or more of the 4 blocks (baseline, hypertonic saline infusion, isotonic saline infusion and baseline 2). Figure 4-10 shows a summary of the occurrences of these units.

The analysis done involved the discrimination of SMUs in all blocks and a comparison between blocks of the following: SMU occurrence, threshold of onset of activity in SMUs, SMU firing rates, occurrence of SMU activity according to the Vicious Cycle Theory and The Pain Adaptation Model, and an analysis of the sequence of recruitment of SMUs.

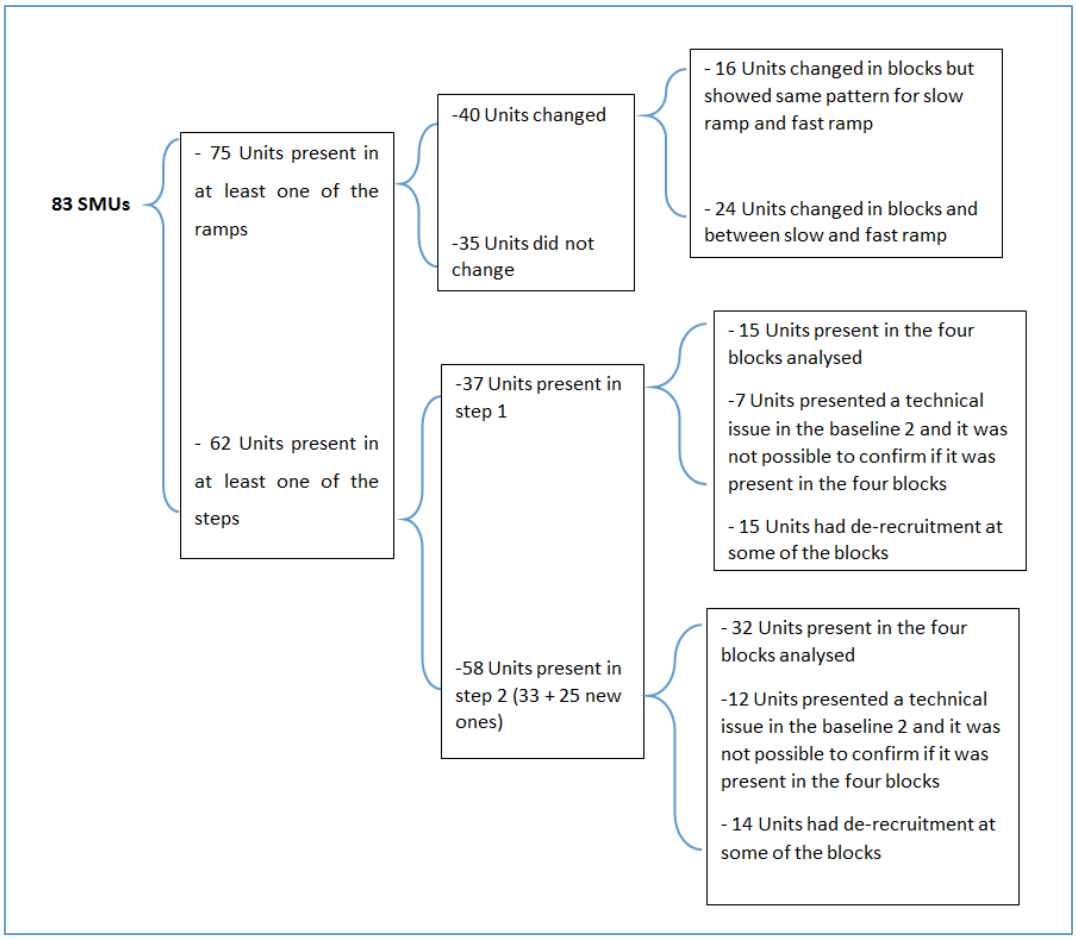


Figure 4-10: Schematic figure to summarize the 83 single motor units characterized for the temporalis muscle in this study. 75 SMUs were present in at least one of the ramp tasks, and of these, 40 SMUs changed their occurrence (i.e. were absent in 1 or more blocks) and 35 did not change (i.e. were present in all blocks). From those 40 that changed, 16 changed in the same way (showed same pattern) for slow ramp and fast ramp and 24 did not change in the same way (changed in blocks and between slow ramp and fast ramp). 62 SMUs present in at least level 1 or level 2 of the 2 step-levels jaw closing task, in which 37 was present in step 1, where 15 units were present in all the blocks, 7 had a technical issue and 15 exhibited a de-recruitment in at least 1 of the blocks and 58 was present in step 2 (33 also present in step 1 + 25 new ones), where 32 units were present in all the blocks, 12 had a technical issue and 14 had a de-recruitment at some of the blocks. Unit = SMU; de-recruitment in a block: SMU not present for that block but was present for 1 or more of the other blocks; “changed” = either recruited in a block and not present in other blocks OR de-recruited in a block but was present in other blocks. “Same pattern” = the recruitment or de-recruitment within one (or more) block (s) happened in the same way for another task.

The EMG activity for the masseter muscle was also analysed for SMU occurrences but the data collected during the baseline 2 block were not analysed in this thesis. Therefore, from the masseter muscle, 58 units were discriminated from the 3 blocks (baseline, hypertonic saline infusion and isotonic saline infusion). Besides the occurrence, a comparison between blocks and analysis of occurrence of SMU activity according to the Vicious Cycle Theory and/or The Pain Adaptation Model was done. Figure 4-11 shows a summary of the occurrence of these units from the masseter muscle.

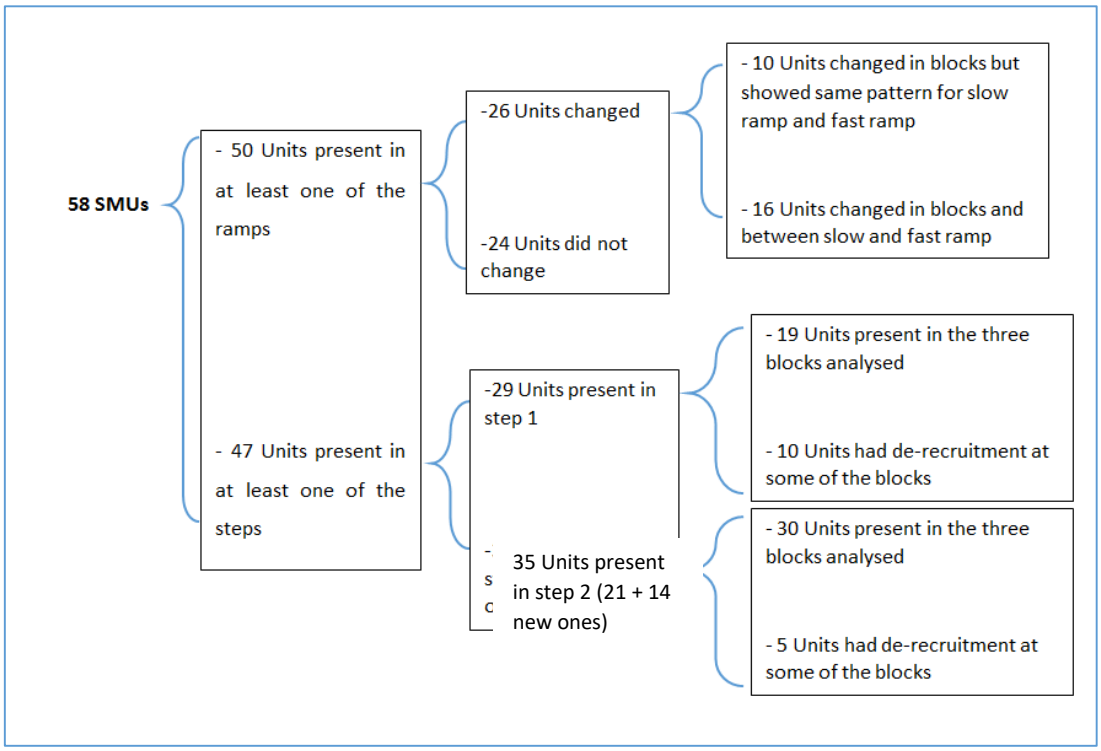


Figure 4-11: Schematic figure to summarize the 58 single motor units characterized for the masseter muscle in this study. 50 SMU present in at least one of the ramps, in which 26 SMUs changed and 24 did not change. From those 26 that changed, 10 changed in the same way (showed same pattern) for slow ramp and fast ramp and 16 did not change in the same way (changed in blocks and between slow ramp and fast ramp). 47 SMUs were present in at least one of the steps, in which 29 were present in step 1 (19 were present in all the blocks analysed and 10 had a de-recruitment for some of the blocks)

and 35 in step 2 (21 also present in step 1 + 14 new ones), where 30 units were present in all the blocks analysed and 5 had a de-recruitment at some of the blocks. Unit = SMU; de-recruitment in a block: SMU not present for that block but was present for 1 or more of the other blocks; “changed” = either recruited in a block and not present in other blocks OR de-recruited in a block but was present in other blocks. “same pattern” = the recruitment or de-recruitment within one (or more) block (s) happened in the same way for another task.

4.4.3 Occurrence of single motor units in the right temporalis muscle:

In total, 83 SMUs were discriminated from the temporalis muscle from 16 participants. Among those 83 SMUs, 75 SMUs were discriminated in at least one of the ramp jaw closing tasks, while 62 (54 also present in the ramps + 8 new SMUs) were discriminated for at least one step level (step 1 and/or step 2) of the 2 step-levels jaw closing task.

There were 8 SMUs (4, 10, 34, 49, 50, 51, 69, 71) that were present exclusively for the 2 step-levels jaw closing tasks, while 21 SMUs (6, 14, 18, 22, 23, 24, 32, 33, 39, 47, 48, 54, 58, 65, 66, 78, 79, 80, 81, 82, 83) were present in the ramp jaw closing tasks but were not present in the 2 step-levels jaw closing tasks. Units 33 and 44 were present in the fast ramp jaw closing task but not in the slow ramp jaw closing task and the units 9, 54, 58 and 70 were present in the slow ramp jaw closing task but not in the fast ramp jaw closing task.

For an easier understanding, the units presented for ramps jaw closing tasks (slow and fast) and the units presented for the 2 step-levels jaw closing task will be described separately in the next sections (section 4.4.3.1 and section 4.4.3.2 respectively).

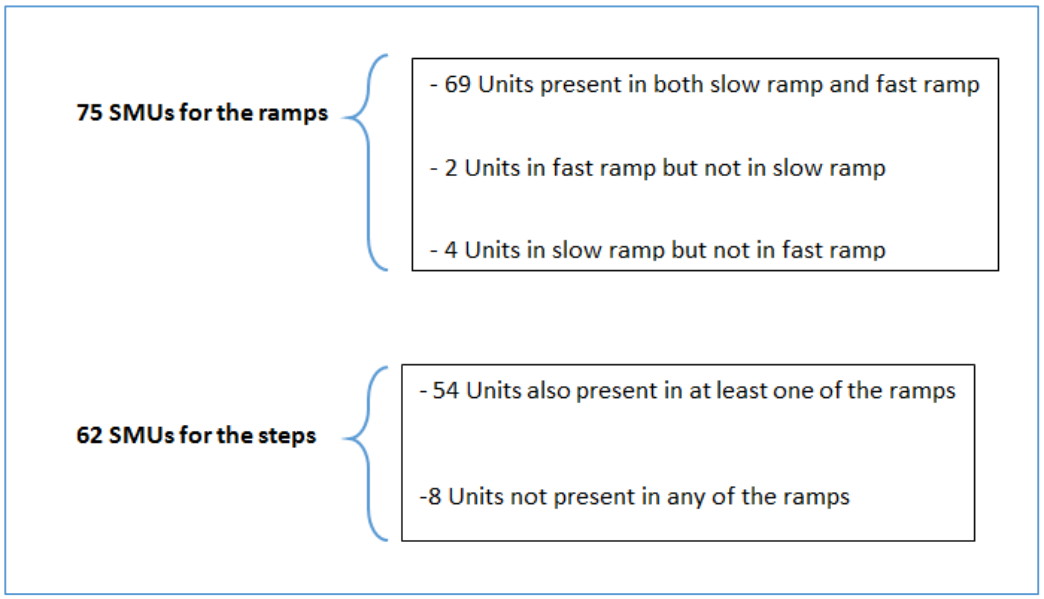


Figure 4-12: Summary of units (i.e. SMUs) in the tasks for the right temporalis muscle. Ramps = slow ramp and fast ramp jaw closing tasks; Steps = Step 1 of the 2 step-levels jaw closing task + Step 2 of the 2 step-levels jaw closing task.

4.4.3.1 Occurrence of motor units in slow ramp and fast ramp tasks for the temporalis muscle:

Table 4-32 lists the occurrences of the 75 SMUs in each recording block (baseline, hypertonic saline infusion block, isotonic saline infusion block, baseline 2) for the ramp tasks (slow and fast ramp jaw closing tasks) in the temporalis muscle. If a SMU was present in at least 2 of the 3 trials done or at least half of trials when more than 3 trials were done, then that SMU was marked as "+" (i.e. present). If not, that SMU was marked as "-" (i.e. not present).

		Slow ramp				Fast Ramp			
Participant	SMU	BS	Hyper	Iso	BS2	BS	Hyper	Iso	BS2
	1	+	+	+	+	+	+	+	+
	2	+	+	+	+	+	+	+	+
	3	-	+	+	+	-	+	+	+
	5	-	+	-	-	-	+	-	-
1	6	-	+	-	-	-	+	-	-
	7	+	+	+	+	+	+	+	+
	8	+	+	+	+	+	-	+	+
2	9	-	+	-	-	-	-	-	-
	11	+	+	+	+	+	+	+	+
	12	+	+	+	+	+	+	+	+
	13	+	+	+	+	+	+	+	+
	14	+	+	+	+	+	+	+	+
4	15	+	+	+	+	+	+	+	+
	16	+	+	+	+	+	+	+	+
	17	+	+	+	+	+	+	+	+
	18	+	-	+	-	+	-	+	-
	19	+	+	+	+	+	+	+	+
	20	+	+	+	+	+	-	+	-
	21	-	+	+	+	-	+	+	+
5	22	-	+	+	-	-	+	+	-
	23	+	+	+	+	+	+	+	+
	24	+	-	-	-	+	-	-	-
	25	+	-	-	+	+	-	-	-
	26	+	+	+	+	+	+	+	+
	27	+	+	+	+	+	-	+	+
6	28	-	+	-	+	-	+	+	+
	29	+	+	+	+	+	+	+	+
	30	+	-	+	+	+	+	+	+
7	31	+	-	-	-	+	-	-	-
	32	+	-	+	+	+	-	+	+
10	33	-	-	-	-	-	-	+	-
	35	+	+	+	+	+	+	+	+
	36	+	+	+	+	+	+	+	+
	37	+	+	+	+	+	+	+	+
	38	+	+	+	+	+	+	+	+
11	39	+	+	+	+	+	+	+	+
	40	+	+	+	+	+	+	+	+
	41	+	+	+	+	-	+	+	+
	42	+	+	+	+	+	+	+	+
	43	-	+	-	-	-	+	-	-
12	44	-	-	-	-	-	+	-	-

		Slow ramp				Fast Ramp			
Participant	SMU	BS	Hyper	Iso	BS2	BS	Hyper	Iso	BS2
	45	+	+	+	+	+	+	+	+
	46	+	+	+	+	+	+	+	+
	47	+	+	+	+	+	+	+	+
13	48	+	+	+	+	+	+	+	+
	52	+	+	TI	TI	+	+	+	+
	53	-	+	TI	TI	+	+	+	+
14	54	-	+	TI	TI	-	-	-	-
	55	+	+	+	+	+	+	+	+
	56	+	+	+	-	+	+	+	+
	57	+	+	+	-	+	+	+	-
	58	-	+	-	-	-	-	-	-
15	59	+	+	+	-	+	+	+	-
	60	+	+	+	+	+	+	+	+
	61	+	+	+	+	-	+	+	-
	62	+	+	-	+	+	-	-	+
	63	+	+	+	+	+	+	+	+
16	64	-	-	+	-	+	+	+	+
	65	+	-	+	-	+	-	+	-
	66	+	+	+	+	+	-	+	-
	67	-	+	+	+	+	+	-	-
	68	-	+	-	+	+	+	-	+
17	70	-	+	-	-	-	-	-	-
	72	+	+	+	+	+	+	+	+
	73	+	+	+	+	+	+	+	+
	74	+	+	+	+	+	+	+	+
18	75	+	+	+	+	+	+	+	+
	76	+	+	+	+	+	+	+	+
	77	+	+	+	+	+	+	+	+
	78	+	+	+	+	+	+	+	+
	79	+	+	+	-	+	-	+	-
	80	-	+	+	+	+	+	+	+
	81	-	+	-	+	-	+	-	+
	82	-	+	-	+	-	+	-	+
19	83	-	+	-	+	-	+	-	+

Table 4-32: All the SMUs (n=75) present in the slow ramp and fast ramp jaw closing tasks under each block. Highlighted are the units that were present in one speed of the slow or fast ramp jaw closing task but which were not present in the other. TI = technical issue in the recording. BS: baseline; hyper: hypertonic saline infusion block; iso: isotonic saline infusion block; BS2: baseline 2.

Among the 75 units present for the ramp jaw closing tasks, 35 SMUs did not change their pattern of occurrence, which means all 35 SMUs were present in both fast and slow ramp jaw closing tasks for all the blocks. Table 4-33 shows those 35 SMUs that were present in all the tasks for the 4 blocks.

In addition, 3 participants (Participant 4, 11 and 18) showed no change in the pattern of recruitment of all the units discriminated from the temporalis muscle in each of those participants. Therefore, these participants presented the same units (SMUs 11, 12, 13, 14, 15 – for participant 4; SMUs 35, 36, 37, 38, 39 for participant 11; and SMUs 72, 73, 74, 75 for participant 18) for both tasks (slow ramp and fast ramp jaw closing tasks) during the four blocks (baseline, hypertonic saline infusion, isotonic saline infusion, baseline 2; see Table 4-33 - highlighted in grey).

		Slow ramp				Fast Ramp			
Participant	SMU	BS	Hyper	Iso	BS 2	BS	Hyper	Iso	BS 2
1	1	+	+	+	+	+	+	+	+
	2	+	+	+	+	+	+	+	+
2	7	+	+	+	+	+	+	+	+
4	11	+	+	+	+	+	+	+	+
	12	+	+	+	+	+	+	+	+
	13	+	+	+	+	+	+	+	+
	14	+	+	+	+	+	+	+	+
	15	+	+	+	+	+	+	+	+
5	16	+	+	+	+	+	+	+	+
	17	+	+	+	+	+	+	+	+
	19	+	+	+	+	+	+	+	+
6	23	+	+	+	+	+	+	+	+
	26	+	+	+	+	+	+	+	+
7	29	+	+	+	+	+	+	+	+
11	35	+	+	+	+	+	+	+	+
	36	+	+	+	+	+	+	+	+
	37	+	+	+	+	+	+	+	+
	38	+	+	+	+	+	+	+	+
	39	+	+	+	+	+	+	+	+
12	40	+	+	+	+	+	+	+	+
	42	+	+	+	+	+	+	+	+
13	45	+	+	+	+	+	+	+	+
	46	+	+	+	+	+	+	+	+
	47	+	+	+	+	+	+	+	+
	48	+	+	+	+	+	+	+	+
15	55	+	+	+	+	+	+	+	+
16	60	+	+	+	+	+	+	+	+
	63	+	+	+	+	+	+	+	+
18	72	+	+	+	+	+	+	+	+
	73	+	+	+	+	+	+	+	+
	74	+	+	+	+	+	+	+	+
	75	+	+	+	+	+	+	+	+
19	76	+	+	+	+	+	+	+	+
	77	+	+	+	+	+	+	+	+
	78	+	+	+	+	+	+	+	+

Table 4-33: SMUs (n=35) that were present in all the tasks for the 4 blocks. In grey, participants who had no change in the pattern of occurrence for all the units discriminated from each of those participants. BS: baseline; hyper: hypertonic saline infusion block; iso: isotonic saline infusion block; BS2: baseline 2.

Of the 75 SMUs, 40 exhibited some change in the pattern of SMU occurrence between the blocks, and of these 40 SMUs, 16 showed the same pattern for slow ramp and fast ramp (n=16) and 24 exhibited some change in the pattern of SMU occurrence between the blocks and between the slow and fast ramp jaw closing tasks (n=24) - See Tables 4-34 and 4-35.

From those 40 SMUs, 16 units (3, 5, 6, 18, 21, 22, 24, 31, 32, 43, 57, 59, 65, 82, 83, 84) showed the same pattern of occurrence for slow and fast ramp jaw closing tasks, and the change in occurrence was only due to the block (yellow highlighted SMUs in Table 4-34). The remaining 24 units (8, 9, 20, 25, 27, 28, 30, 33, 41, 44, 52, 53, 54, 56, 58, 61, 62, 64, 66, 67, 68, 70, 79, 80) had different patterns of occurrence for the different tasks as well as changes in occurrence between the blocks. Two units (33 and 44) were found in the slow ramp jaw closing task, but not in the fast ramp jaw closing task, while 4 units (9, 54, 58 and 70) were found in the fast ramp jaw closing task but not in the slow ramp jaw closing task.

For participant 14, it was possible to discriminate 3 units (52, 53, and 54). However, due to technical issues (noisy signal), the data from the slow ramp jaw closing tasks for the block of Isotonic saline infusion and baseline 2 were lost. These units are still mentioned in this thesis as unit 54 was present in hypertonic saline infusion slow ramp jaw closing task but not hypertonic saline infusion fast ramp jaw closing task, while unit 52 was present in all the other tasks and unit 53 was not present in baseline slow ramp jaw closing task but it was present in baseline fast ramp jaw closing task.

		Slow ramp				Fast Ramp			
Participant	SMU	BS	Hyper	Iso	BS 2	BS	Hyper	Iso	BS 2
1	3	-	+	+	+	-	+	+	+
	5	-	+	-	-	-	+	-	-
	6	-	+	-	-	-	+	-	-
2	8	+	+	+	+	+	-	+	+
	9	-	+	-	-	-	-	-	-
5	18	+	-	+	-	+	-	+	-
	20	+	+	+	+	+	-	+	-
	21	-	+	+	+	-	+	+	+
	22	-	+	+	-	-	+	+	-
6	24	+	-	-	-	+	-	-	-
	25	+	-	-	+	+	-	-	-
	27	+	+	+	+	+	-	+	+
	28	-	+	-	+	-	+	+	+
7	30	+	-	+	+	+	+	+	+
	31	+	-	-	-	+	-	-	-
10	32	+	-	+	+	+	-	+	+
	33	-	-	-	-	-	-	+	-
12	41	+	+	+	+	-	+	+	+
	43	-	+	-	-	-	+	-	-
	44	-	-	-	-	-	+	-	-
14	52	+	+	TI	TI	+	+	+	+
	53	-	+	TI	TI	+	+	+	+
	54	-	+	TI	TI	-	-	-	-
15	56	+	+	+	-	+	+	+	+
	57	+	+	+	-	+	+	+	-
	58	-	+	-	-	-	-	-	-
	59	+	+	+	-	+	+	+	-
16	61	+	+	+	+	-	+	+	-
	62	+	+	-	+	+	-	-	+
	64	-	-	+	-	+	+	+	+
17	65	+	-	+	-	+	-	+	-
	66	+	+	+	+	+	-	+	-
	67	-	+	+	+	+	+	-	-
	68	-	+	-	+	+	+	-	+
	70	-	+	-	-	-	-	-	-
19	79	+	+	+	-	+	-	+	-
	80	-	+	+	+	+	+	+	+
	81	-	+	-	+	-	+	-	+
	82	-	+	-	+	-	+	-	+
	83	-	+	-	+	-	+	-	+

Table 4-34: Shows only those SMUs (n=40) that exhibited some change in the pattern of occurrence between blocks (n=16; yellow highlighted SMUs), or between the blocks and between the slow and fast ramp jaw closing tasks (n=24). TI = technical issue in the recording. BS: baseline; hyper: hypertonic saline infusion block; iso: isotonic saline infusion block; BS2: baseline 2.

		Slow ramp				Fast Ramp			
Participant	SMU	BS	Hyper	Iso	BS 2	BS	Hyper	Iso	BS 2
1	3	-	+	+	+	-	+	+	+
	5	-	+	-	-	-	+	-	-
	6	-	+	-	-	-	+	-	-
5	18	+	-	+	-	+	-	+	-
	21	-	+	+	+	-	+	+	+
	22	-	+	+	-	-	+	+	-
6	24	+	-	-	-	+	-	-	-
7	31	+	-	-	-	+	-	-	-
10	32	+	-	+	+	+	-	+	+
12	43	-	+	-	-	-	+	-	-
15	57	+	+	+	-	+	+	+	-
	59	+	+	+	-	+	+	+	-
17	65	+	-	+	-	+	-	+	-
19	81	-	+	-	+	-	+	-	+
	82	-	+	-	+	-	+	-	+
	83	-	+	-	+	-	+	-	+

Table 4-35: Shows only those units (n=16) that did not exhibit a change in the pattern of occurrence across all 4 blocks when compared between the slow ramp and fast ramp jaw closing tasks. BS: baseline; hyper: hypertonic saline infusion block; iso: isotonic saline infusion block; BS2: baseline 2.

According to Table 4-35, it is possible to notice that some units were present or were not present within the hypertonic saline infusion block. Units 5, 6 and 43, for example were present only during the hypertonic saline infusion block for slow and fast ramp jaw closing tasks. Units 81, 82 and 83 were present for the slow and fast ramp jaw closing tasks during hypertonic saline infusion and baseline 2 blocks.

Units 18, 32, 65 were not present in the hypertonic saline infusion block; unit 32 was present in all the other blocks, and units 18 and 65 were present in all the other blocks but not for baseline 2.

Units 3, 21 and 22 were not present in the baseline, but were at least present in all the other saline infusion blocks.

Unit 24 and 31 were present only in baseline, but were not present after the injection of the first solution (isotonic and hypertonic consequently) and remained not present for the other blocks.

And lastly, units 57 and 59 were present in all the blocks but were not present in the baseline 2.

For all the other participants (Participant 2, 5, 6, 7, 10, 12, 14, 15, 16, 17, 19), it was possible to note some change in the pattern of occurrence of units not only within a block, but also with different patterns between slow ramp and fast ramp (n=24; Table 4-36). The solution applied first on these participants is also showed in this table.

		Slow ramp				Fast Ramp			
Participant	SMU	BS	Hyper	Iso	BS 2	BS	Hyper	Iso	BS 2
2 (I)	8	+	+	+	+	+	-	+	+
	9	-	+	-	-	-	-	-	-
5 (H)	20	+	+	+	+	+	-	+	-
6 (I)	25	+	-	-	+	+	-	-	-
	27	+	+	+	+	+	-	+	+
	28	-	+	-	+	-	+	+	+
7 (H)	30	+	-	+	+	+	+	+	+
10 (I)	33	-	-	-	-	-	-	+	-
12 (I)	41	+	+	+	+	-	+	+	+
	44	-	-	-	-	-	+	-	-
14 (I)	52	+	+	TI	TI	+	+	+	+
	53	-	+	TI	TI	+	+	+	+
	54	-	+	TI	TI	-	-	-	-
15 (H)	56	+	+	+	-	+	+	+	+
	58	-	+	-	-	-	-	-	-
16 (H)	61	+	+	+	+	-	+	+	-
	62	+	+	-	+	+	-	-	+
	64	-	-	+	-	+	+	+	+
17 (I)	66	+	+	+	+	+	-	+	-
	67	-	+	+	+	+	+	-	-
	68	-	+	-	+	+	+	-	+
	70	-	+	-	-	-	-	-	-
19 (I)	79	+	+	+	-	+	-	+	-
	80	-	+	+	+	+	+	+	+

Table 4-36: Shows only those units (n=24) that exhibited a change in the pattern of occurrence between slow ramp and fast ramp. Letter in brackets indicates the solution applied first for that participant and is noted after the participant's number as (H) for hypertonic saline being applied first and (I) when the isotonic solution was applied first. TI = technical issue in the recording. BS: baseline; hyper: hypertonic saline infusion block; iso: isotonic saline infusion block; BS2: baseline 2.

Units 9, 58 and 70 were present only during hypertonic saline infusion slow ramp jaw closing tasks but were not present in any of the blocks for the fast ramp jaw closing tasks. Unit 44, on the other hand, was present only for the hypertonic saline infusion fast ramp jaw closing task but not in any of the other ramp jaw closing tasks.

Unit 30 was not present during hypertonic saline infusion slow ramp jaw closing task but was present for other blocks and tasks, and unit 79 was not present in the hypertonic saline infusion fast ramp jaw closing task but was for the hypertonic saline infusion slow ramp jaw closing task.

Figure 4-13 summarizes schematically the 75 SMUs discriminated for the ramps jaw closing tasks in this study.

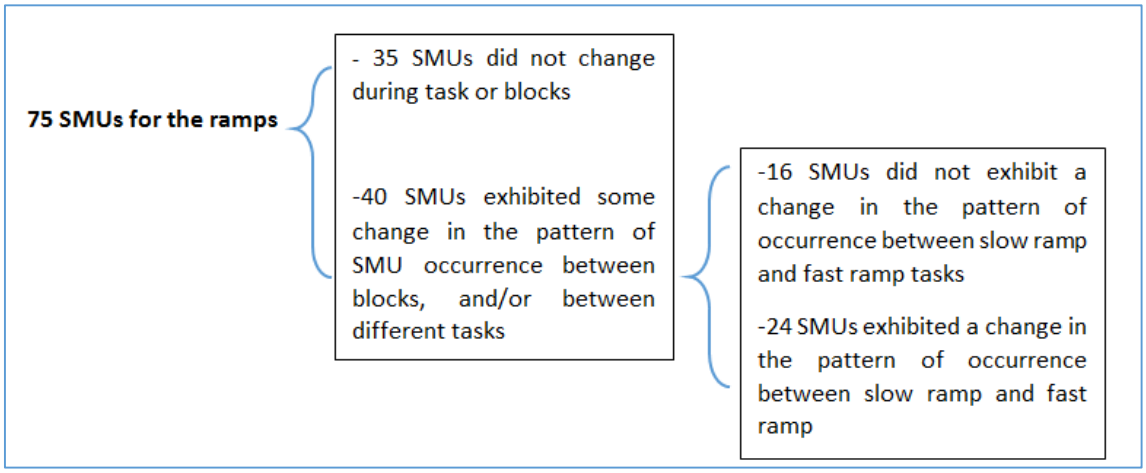


Figure 4-13: Schematic figure to summarize the 75 SMUs discriminated for the ramps tasks in the right temporalis muscle in this study and changes in SMU occurrences or not in the blocks. Unit = SMU.

4.4.3.2 *Occurrence of motor units in steps for temporalis muscle:*

The 62 motor units present in the 2 step-levels jaw closing tasks are described in Table 4-37. Of the 62 SMUs, 54 were also present in one or more of the ramp jaw closing tasks and the remaining 8 were new SMUs that were only present in the 2 step-levels jaw closing task.

		Step 1					Step 2			
Participant	SMU	BS	Hyper	Iso	BS 2		BS	Hyper	Iso	BS 2
	1	+	+	+	+		+	+	+	+
	2	+	+	+	+		+	+	+	+
	3	+	-	+	-		+	-	+	+
	4	-	-	-	-		+	-	+	-
1	5	-	+	-	-		-	+	-	+
	7	+	+	+	TI		+	+	+	TI
	8	-	-	-	TI		+	+	+	TI
	9	+	+	+	TI		+	+	+	TI
2	10	-	-	-	TI		+	+	+	TI
	11	+	+	+	TI		+	+	+	TI
	12	+	+	+	TI		+	+	+	TI
	13	+	+	+	TI		+	+	+	TI
4	15	+	+	+	TI		+	+	+	TI
	16	+	+	+	+		+	+	+	+
	17	-	-	-	-		+	+	+	+
	19	-	-	-	-		+	+	+	+
	20	-	-	-	-		+	+	+	+
5	21	-	-	-	-		+	+	+	+
	25	-	-	-	-		+	+	+	+
	26	+	+	+	+		+	+	+	+
	27	-	-	+	+		+	+	+	+
6	28	-	-	-	-		+	+	+	+
	29	+	+	+	+		+	+	+	+
	30	+	+	+	-		+	+	+	+
7	31	-	-	-	-		-	+	+	-
10	34	-	-	-	-		+	+	+	+
	35	+	+	+	+		+	?	?	+
11	36	+	+	+	-		+	+	+	+

		Step 1					Step 2			
	SMU	BS	Hyper	Iso	BS 2		BS	Hyper	Iso	BS 2
11	37	-	-	-	-		+	+	+	+
	38	-	-	-	-		+	+	+	-
12	40	+	+	+	+		+	+	+	+
	41	-	+	-	-		+	+	+	+
	42	+	+	+	-		+	+	+	+
	43	-	+	-	-		-	+	-	-
	44	-	+	-	-		-	+	-	-
13	45	+	+	+	+		?	?	?	?
	46	+	+	+	+		?	?	?	?
	49	+	+	+	+		?	?	?	?
	50	-	-	-	-		+	-	-	-
	51	-	-	-	-		-	+	+	+
14	52	-	-	-	-		+	+	+	+
	53	-	-	-	-		+	+	+	+
15	55	+	+	+	TI		+	+	+	TI
	56	-	-	-	TI		+	+	+	TI
	57	-	-	-	TI		+	+	+	TI
	59	-	-	-	TI		+	+	+	TI
16	60	+	+	+	+		+	+	+	+
	61	-	-	-	+		-	+	-	+
	62	+	-	-	-		+	+	+	+
	63	+	-	+	-		+	+	+	+
	64	-	-	-	-		+	-	+	+
17	67	-	-	-	-		-	+	-	-
	68	+	+	-	-		+	+	+	+
	69	+	+	-	-		+	+	+	+
	70	-	-	-	-		+	-	+	+
	71	-	-	-	-		-	+	-	+
18	72	+	+	+	+		+	+	+	+
	73	+	+	+	+		+	+	+	+
	74	+	+	+	+		+	+	+	+
	75	+	+	+	+		+	+	+	+
19	76	+	-	+	-		+	+	+	+
	77	-	-	-	-		+	+	+	+

Table 4-37: Motor units (n=62) discriminated in 2 step-levels jaw closing tasks. In grey, all the motor units that were exclusively found for steps tasks and were not present in the ramp tasks. TI: technical issue. ? = when it was not possible to confirm the presence or absence of the unit for that task. BS: baseline; hyper: hypertonic saline infusion block; iso: isotonic saline infusion block; BS2: baseline 2.

From the 62 units described in Table 4-37, 37 units were discriminated for the step 1 and 58 units were discriminated for the step 2; these 58 were made up of 33 present for step 1 plus an additional 25 newly recruited SMUs. Due to the greater force and higher level of EMG activity for step 2, it was not possible to confirm precisely if 4 small units (35, 45, 46, and 49) were present or absent. For those cases, a question mark is used in the table.

For the step 1 of the 2 step-levels jaw closing task, from the 37 units discriminated, 15 units (unit 1, 2, 16, 26, 29, 35, 40, 45, 46, 49, 60, 72, 73, 74, 75) were present in all the four blocks, 15 units (unit 3, 5, 27, 30, 36, 41, 42, 43, 44, 61, 62, 63, 68, 69, 76) were not present in some of the blocks and for 7 units (unit 7, 9, 11, 12, 13, 15, 55) it was not possible to confirm if they were present in all of the blocks due to a technical issue (see Methods).

For the step 2 of the 2 step-levels jaw closing task, from the 58 units discriminated, 32 units (unit 1, 2, 16, 17, 19, 20, 21, 25, 26, 27, 28, 29, 30, 34, 36, 37, 40, 41, 42, 52, 53, 60, 62, 63, 68, 69, 72, 73, 74, 75, 76, 77) were present in all of the four blocks, while 14 units (unit 3, 4, 5, 31, 38, 43, 44, 50, 51, 61, 64, 67, 70, 71) were not present in some of the blocks. For 12 units (unit 7, 8, 9, 10, 11, 12, 13, 15, 55, 56, 57, 59) it was not possible to confirm if they were present in all of the blocks due to a technical issue (see Methods) or due to these units being obscured by the increased EMG activity accompanying the greater force on the level 2 – See figure -4-15.

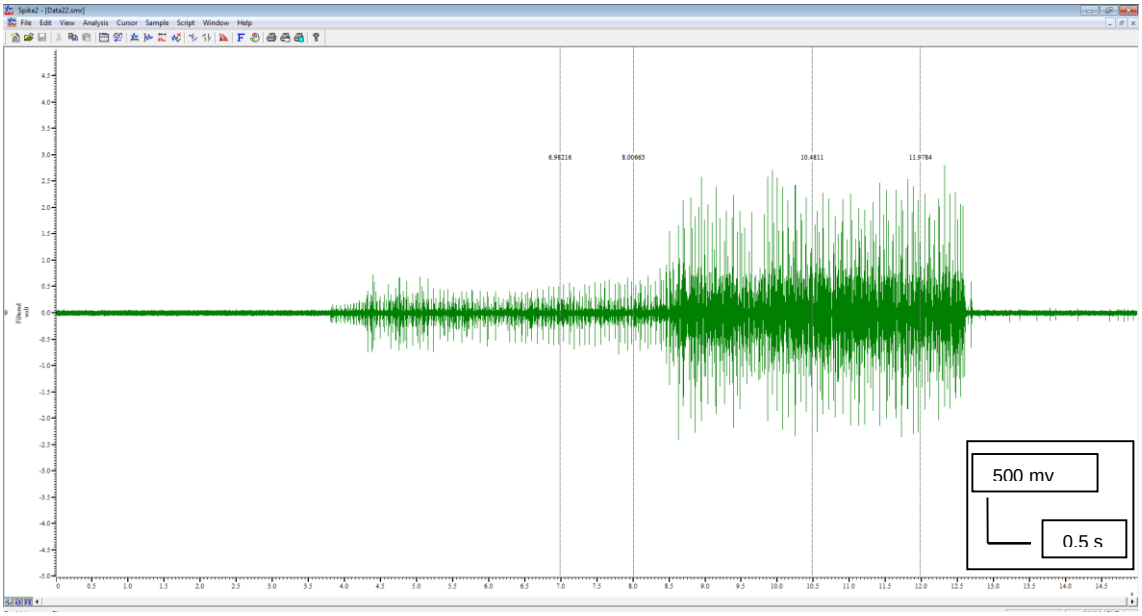


Figure 4-14: Example of temporalis EMG activity recorded during the 2 step-levels jaw closing task. Note that the small units become obscured by the increased EMG activity accompanying the greater force at the level 2, and therefore they can be difficult or impossible to discriminate.

Figure 4-15 summarizes the 62 SMUs discriminated for the 2 step-levels jaw closing tasks in the right temporalis muscle in this study.

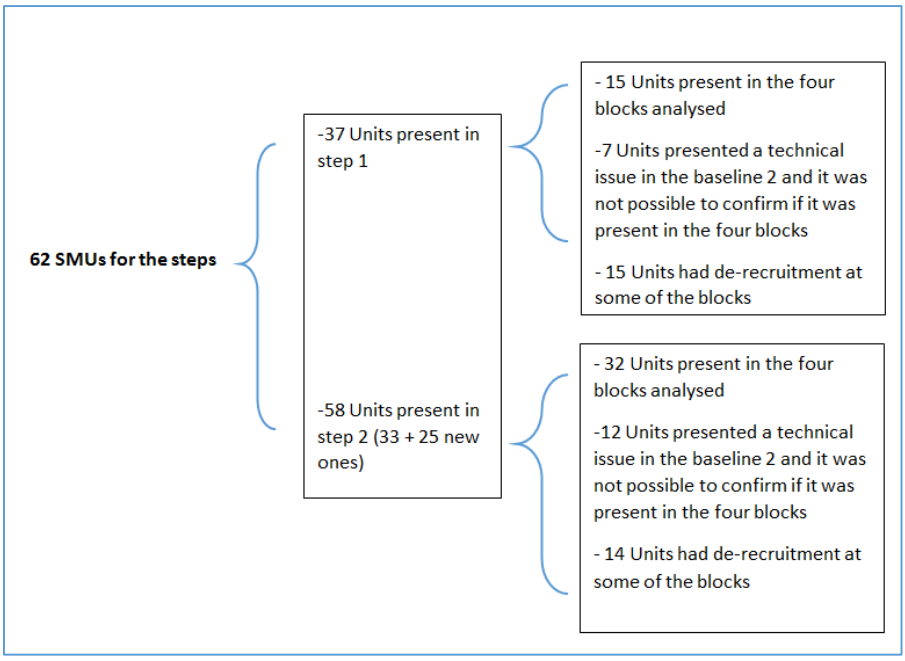


Figure 4-16: Schematic figure to summarize the 62 SMUs discriminated for the 2 step-levels jaw closing tasks in the right temporalis muscle in this study. Unit = SMU.

4.4.3.3 Comparisons between blocks:

For each of the slow ramp and fast ramp jaw closing tasks, and step 1 and step 2 of the 2 steps-levels jaw closing task, the hypertonic saline infusion block (H) was compared with each of the other blocks - baseline (B), Isotonic saline infusion (I) and Baseline 2 (BS 2) - in terms of the number of units during the hypertonic saline infusion block of tasks that

- Exhibited no change in the pattern of occurrence between the 2 blocks,
- Became present in the hypertonic saline infusion block (i.e. was recruited), or
- Was not present (i.e. was de-recruited) during the hypertonic saline infusion block.

These analyses are shown for the baseline vs. hypertonic saline block in Table 4-38, the isotonic saline vs. hypertonic saline block in Table 4-39 and the baseline 2 vs. hypertonic saline block in Table 4-40.

	B x H SR	B x H FR	B x H step 1	B x H step 2
No change	48/73 (65.8%)	46/71 (64.8%)	29/37 (78.4%)	45/58 (77.6%)
Recruited	18/73 (24.6%)	13/71 (18.3%)	4/37 (10.8%)	8/58 (13.8%)
De-recruited	7/73 (9.6%)	12/71 (16.9%)	4/37 (10.8%)	5/58 (8.6%)

Table 4-38: Describes the comparison between the hypertonic saline infusion block with the baseline block for slow ramp, fast ramp, step 1 and step 2 of the 2-steps levels jaw closing tasks. For the slow ramp tasks, 65.8% of the units did not change, 64.8% of the units did not change for the fast ramp, and 78.4% and 77.6% of the units did not change for step 1 and step 2 respectively. Recruited = SMU became present in the hypertonic saline infusion block; de-recruited = SMU was not present during the hypertonic saline infusion block but was present in baseline.

	I x H SR	I x H FR	I x H step 1	I x H step 2
No change	53/70 (75.7%)	53/71 (74.6%)	27/37 (73%)	48/58 (82.8%)
Recruited	12/70 (17.1%)	9/71 (12.7%)	6/37 (16.2%)	6/58 (10.3%)
De-recruited	5/70 (7.2%)	9/71 (12.7%)	4/37 (10.8%)	4/58 (6.9%)

Table 4-39: Describes the comparison between the hypertonic saline infusion block with the isotonic saline infusion block for slow ramp, fast ramp, step 1 and step 2 jaw closing tasks. For the slow ramp tasks, 75.7% of the units did not change, 74.6% of the units did not change for the fast ramp, and 73% and 82.8% of the units did not change for step 1 and step 2 respectively. Recruited = SMU became present in the hypertonic saline infusion block; de-recruited = SMU was not present during the hypertonic saline infusion block

	BS 2 x H SR	BS 2 x H FR	BS2 x H step 1	BS2 x H step 2
No change	56/70 (80%)	58/71(81.7%)	19/30 (63.3%)	38/46 (82.6%)
Recruited	11/70 (15.7%)	9/71 (12.7%)	9/30 (30%)	5/46 (10.8%)
De-recruited	3/70 (4.3%)	4/71 (5.6%)	2/30 (6.7%)	3/46 (6.5%)

Table 4-40: Describes the comparison between the hypertonic block with the baseline 2 block for slow ramp, fast ramp, step 1 and step 2 jaw closing tasks. A few units were excluded from step tasks due to technical issues. For the slow ramp tasks, 80% of the units did not change, 81.7% of the units did not change for the fast ramp, and 63.3% and 82.6% of the units did not change for step 1 and step 2 respectively. Recruited = SMU became present in the hypertonic saline infusion block; de-recruited = SMU was not present during the hypertonic saline infusion block

Figure 4-17 shows an example of the recruitment of SMUs during the hypertonic saline infusion block.

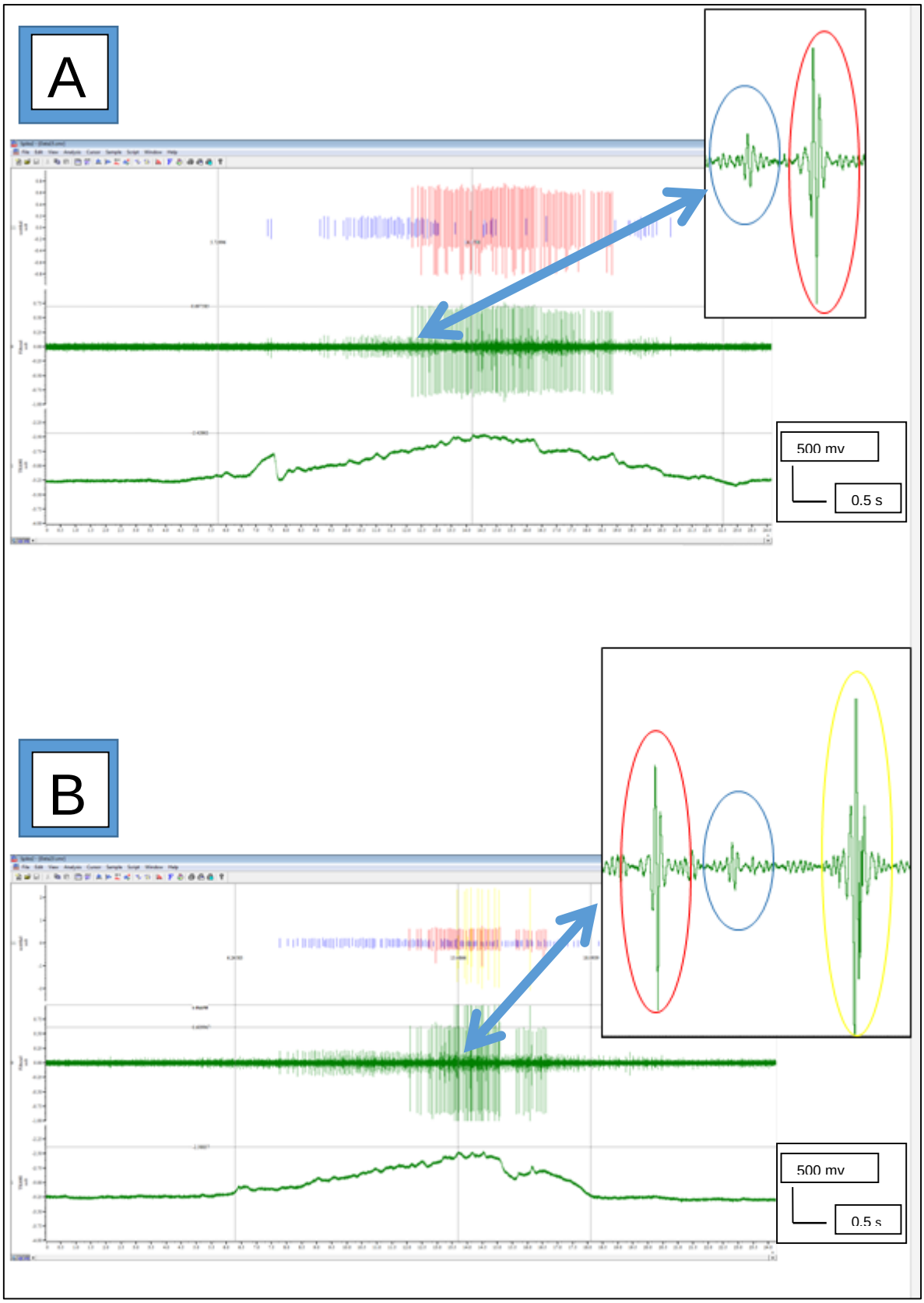


Figure 4-17: Shows the recruitment of a new unit. A: Slow ramp jaw closing task during isotonic saline block and showing the recruitment of 2 SMUs. B: Slow ramp jaw closing task during hypertonic saline

block and showing the recruitment of 3 SMUs. New SMU in yellow recruited in the hypertonic saline block but was not present in the isotonic saline block. X-axis: time in seconds (Sec). Y-axis: force transducer output in volts (V).

4.4.3.4 Temporalis SMU activity and the vicious cycle theory and the pain adaptation model

An analysis was performed to determine whether the change in the pattern of occurrence of SMUs recorded in the temporalis muscle could be explained on the basis of the principles outlined for the Vicious Cycle Theory or the Pain Adaptation Model. This analysis is summarized in Table 4-41 and was performed only for the comparison between the isotonic saline block and the hypertonic saline block.

If the SMU was recruited during the hypertonic saline infusion block but was inactive (i.e. not present) in the isotonic saline infusion block, then the pattern of occurrence of the SMU was considered to be consistent with the Vicious Cycle Theory as the pain, according to this theory, would cause “muscle hyperactivity”. On the other hand, if the SMU becomes inactive (i.e. not present) during the hypertonic saline infusion block in comparison with the isotonic saline infusion block, this pattern of occurrence was considered to be consistent with the Pain Adaptation Model which proposes decreased agonist muscle activity in pain so as to result in slower and smaller movements to prevent further injury and help healing.

This analysis, however, only considered the presence or absence or the unit and did not consider the possible effect of the pain on the firing rates of those units that were present in the hypertonic saline infusion block as well as the isotonic saline infusion block.

		Slow ramp		Fast Ramp		Step 1		Step 2	
Participant	SMU	VCT	PAM	VCT	PAM	VCT	PAM	VCT	PAM
1	1								
	2								
	3						X		X
	4	-	-	-	-	-	-		X
	5	X		X		X		X	
	6	X		X		-	-	-	-
2	7								
	8				X	-	-		
	9	X		-	-				
	10	-	-	-	-	-	-		
4	11								
	12								
	13								
	14					-	-	-	-
	15								
5	16								
	17					-	-		
	18		X		X	-	-	-	-
	19					-	-		
	20				X	-	-		
	21					-	-		
	22					-	-	-	-
6	23					-	-	-	-
	24					-	-	-	-
	25					-	-		
	26								
	27				X		X		
	28	X				-	-		

		Slow ramp		Fast Ramp		Step 1		Step 2	
Participant	SMU	VCT	PAM	VCT	PAM	VCT	PAM	VCT	PAM
7	29								
	30		X						
	31					-	-		
10	32		X		X	-	-	-	-
	33	-	-		X	-	-	-	-
	34	-	-	-	-	-	-		
11	35							-	-
	36								
	37					-	-		
	38					-	-		
	39					-	-	-	-
12	40								
	41					X			
	42								
	43	X		X		X		X	
	44	-	-	X		X		X	
13	45							-	-
	46							-	-
	47					-	-	-	-
	48					-	-	-	-
	49	-	-	-	-			-	-
	50	-	-	-	-	-	-		
	51	-	-	-	-	-	-		
14	52	LOST				-	-		
	53	LOST				-	-		
	54	LOST		-	-	-	-	-	-
15	55								
	56					-	-		
	57					-	-		
	58	X		-	-	-	-	-	-
	59					-	-		
16	60								
	61							X	
	62	X							
	63						X		
	64		X			-	-		X

		Slow ramp		Fast Ramp		Step 1		Step 2	
Participant	SMU	VCT	PAM	VCT	PAM	VCT	PAM	VCT	PAM
17	65		X		X	-	-	-	-
	66				X	-	-	-	-
	67			X		-	-	X	
	68	X		X		X			
	69	-	-	-	-	X			
	70	X		-	-	-	-		X
	71	-	-	-	-	-	-	X	
18	72								
	73								
	74								
	75								
19	76						X		
	77					-	-		
	78					-	-	-	-
	79				X	-	-	-	-
	80					-	-	-	-
	81	X		X		-	-	-	-
	82	X		X		-	-	-	-
	83	X		X		-	-	-	-
total		12	5	9	9	6	4	6	4

Table 4-41: Shows the 83 units discriminated from the temporalis muscle in this study. One “x” was marked when the pattern of recruitment of a certain unit was supportive of one of the models (VCT or PAM), the sign “-“ was used when the unit was not present for that specific block, and nothing was marked when the unit was present but its occurrence did not support either of those models. Note that the majority of the units were neither consistent with the Vicious Cycle Theory nor the Pain Adaptation Model.

According to Table 4-41, it is possible to notice that 12 units supported the Vicious Cycle Theory for the slow ramp jaw closing tasks, 9 units for the fast ramp jaw closing tasks and 6 units for each of step 1 and step 2 of the 2 step-levels jaw closing tasks. On the other hand, 5 units supported the Pain Adaptation model on the slow ramp jaw closing tasks, 9 units for the fast ramp jaw closing tasks, and lastly 4 units supported

the Pain Adaptation Model for each of step 1 and 2 of the 2 step-levels jaw closing task.

A further analysis was done separately according to the type of task and the number of SMUs recorded per participant, and whether the patterns of recruitments and de-recruitments of all SMUs recorded at a site supported the Vicious Cycle Theory, the Pain Adaptation Model or neither of those models. This analysis is shown in Tables 4-42, 4-43, 4-44, 4-45.

From the 70 SMUs studied in the slow ramp jaw closing task (table 4-42), 80% (n=56) of the units did not support either the Vicious Cycle Theory nor the Pain Adaptation Model, and only 17.1% (n=12) supported the proposals of the VCT and 7.1% (n=5) supported the proposals of the PAM.

Slow ramp: (Units n= 70)

Participant	Number of Units	VCT	PAM	Neither
1	5	2/5		3/5
2	3	1/3		2/3
4	5			5/5
5	7		1/7	6/7
6	6	1/6		5/6
7	3		1/3	2/3
10	1		1/1	1/1
11	5			5/5
12	4	1/4		3/4
13	4			4/4
14		TI	TI	TI
15	5	1/5		4/5
16	5	1/5	1/5	3/5
17	5	2/5	1/5	2/5
18	4			4/4
19	8	3/8		5/8
Total	70	12	5	56

Table 4-42: Units present for the slow ramp task and consistency or not with the Vicious Cycle Theory (VCT) and the Pain Adaptation Model (PAM). From the 70 units presented for this test, 80% (n= 56) of the units exhibited a pattern of occurrence that did not support either the Vicious Cycle Theory nor the Pain Adaptation Model, and only 17.1% (n=12) supported the proposals of the VCT and 7.1% (n=5) supported the proposals of the PAM.

From the 71 units studied during the fast ramp jaw closing task (Table 4-43), 75.7% (n= 53) of the units did not support either of the Vicious Cycle Theory nor the Pain Adaptation Model, and only 12.8% (n=9) supported the proposals of the VCT and 12.8% (n=9) supported the proposals of the PAM.

Fast ramp: (Units n= 71)

Participant	Number of Units	VCT	PAM	Neither
1	5	2/5		3/5
2	2		1/2	1/2
4	5			5/5
5	7		2/7	5/7
6	6		1/6	5/6
7	3			3/3
10	2		2/2	0/2
11	5			5/5
12	5	2/5		3/5
13	4			4/4
14	2			2/2
15	4			4/4
16	5			5/5
17	4	2/4	2/4	0/4
18	4			4/4
19	8	3/8	1/8	4/8
Total	71	9	9	53

Table 4-43: Units presents for the fast ramp task and consistency or not with the Vicious Cycle Theory (VCT) and the Pain Adaptation Model (PAM). From the 71 units presents for this test, 75.7% (n= 53) of the units exhibited a pattern of occurrence that did not support either of the Vicious Cycle Theory nor the Pain Adaptation Model, and only 12.8% (n=9) supported the proposals of the VCT and 12.8% (n=9) supported the proposals of the PAM.

From the 37 units present for the step 1 of the 2 step-levels jaw closing task (Table 4-44), 73% (n=27) of the units did not support either of the Vicious Cycle Theory nor the Pain Adaptation Model, and only 16.2% (n=6) supported the proposals of the VCT and 10.8% (n=4) supported the proposals of the PAM.

Step 1: (Units n = 37)

Participant	Number of Units	VCT	PAM	Neither
1	4	1/4	1/4	2/4
2	2			2/2
4	4			4/4
5	1			1/1
6	2		1/2	1/2
7	2			2/2
10	0			0/0
11	2			2/2
12	5	3/5		2/5
13	3			3/3
14	0			0/0
15	1			1/1
16	4		1/4	3/4
17	2	2/2		0/2
18	4			4/4
19	1		1/1	0/1
Total	37	6	4	27

Table 4-44: Units presents for the step 1 of the 2 step-levels jaw closing task and consistency or not with the Vicious Cycle Theory (VCT) and the Pain Adaptation Model (PAM). From the 37 units presents for this test, 73% (n= 27) of the units exhibited a pattern of occurrence that did not support either of the Vicious Cycle Theory nor the Pain Adaptation Model, and only 16.2% (n=6) supported the proposals of the VCT and 10.8% (n=4) supported the proposals of the PAM.

From the 58 units present for step 2 of the 2 step-levels jaw closing task (Table 4-45), 82.8% (n= 48) of the units did not support either of the Vicious Cycle Theory nor the

Pain Adaptation Model, and only 10.3% (n=6) supported the proposals of the VCT and 6.9% (n=4) supported the proposals of the PAM.

Step 2: (Units n= 58)

Participant	Number of Units	VCT	PAM	Neither
1	5	1/5	2/5	2/5
2	4			4/4
4	4			4/4
5	5			5/5
6	4			4/4
7	3			3/3
10	1			1/1
11	3			3/3
12	5	2/5		3/5
13	2			2/2
14	2			2/2
15	4			4/4
16	5	1/5	1/5	3/5
17	5	2/5	1/5	2/5
18	4			4/4
19	2			2/2
Total	58	6	4	48

Table 4-45: Units presents for the step 2 of the 2 step-levels jaw closing task and consistency or not with the Vicious Cycle Theory (VCT) and the Pain Adaptation Model (PAM). From the 58 units presents for this test, 82.8% (n=48) of the units exhibited a pattern of occurrence that did not support either of the Vicious Cycle Theory nor the Pain Adaptation Model, and only 10.3% (n=6) supported the proposals of the VCT and 6.9% (n=4) supported the proposals of the PAM.

4.4.3.5 *Threshold analysis of temporalis SMU activity*

A repeated measures analysis of variance (ANOVA) of all discriminated SMU threshold values for each jaw task across baseline, hypertonic saline and isotonic saline infusion blocks was performed to determine the main effect of repeating the jaw task and also if there was any effect of the infusing saline into the masseter muscle.

The results showed no significant effect on the threshold values of repeating the task during the baseline ($p=0.89$) and isotonic saline infusion ($p=0.52$) of the slow ramp jaw closing task, but showed a significant difference on repetitions for the hypertonic saline infusion block ($p=0.7$). For the fast ramp jaw closing task, no significant difference was found during the hypertonic saline block ($p=0.65$) but significant differences between repetitions was found during the baseline block ($p=0.006$) and the isotonic saline block ($p=0.016$).

The overall mean of the thresholds for all the SMUs was calculated with multiple comparisons with Bonferroni corrections for each block in order to determine if there was an effect of block on SMU threshold for the slow ramp and fast ramp jaw closing tasks and no significant difference was found for either of the tasks ($p>0.05$).

Of the 75 SMUs studied in the slow ramp and fast ramp jaw closing tasks (see Figure 4-18, 4-19), a total of 37 SMUs were able to be assessed for threshold analysis.

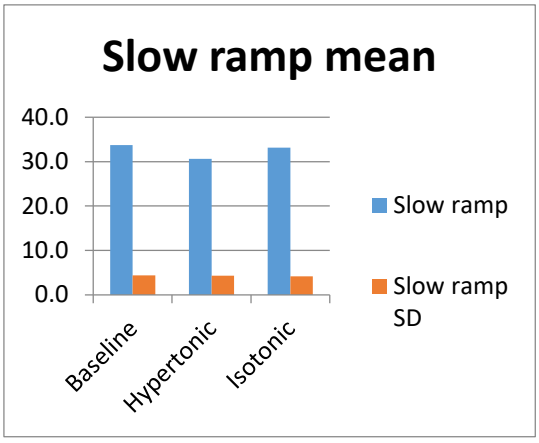


Figure 4-18: Mean and SD (N) for threshold of onset of SMU activity in the slow ramp jaw closing task.

For the slow ramp task it is possible to notice that the hypertonic threshold overall mean was lower compared with the other blocks, however, the main effect of session (baseline, hypertonic infusion, isotonic infusion) was not statistically significant ($p>0.05$) across all threshold values for the slow ramp tasks.

A further analysis was done for each unit individually in order to determine if, even with no change in the overall activity, some units would possibly show an effect on their individual threshold values. This individual SMU analysis is shown in Table 4-46.

SMU	Baseline	Hypertonic	Isotonic	B SD	H SD	I SD
11	22.0	24.6	23.1	0	7.1	3.4
12	29.5	27.0	23.5	5.6	6.0	0.8
13	46.8	49.4	39.6	5.6	1.3	5.4
14	28.5	24.8	22.3	7.3	6.7	1.7
15	40.1	35.2	29.0	4.1	8.7	4.7
16	20.0	24.7	28.1	3.0	5.5	7.8
17	45.2	43.0	50.5	1.7	2.8	1.9
18	44.0	51.7	58.4	2.8	3.3	1.0
19	49.4	42.7	48.8	2.2	1.9	5.6
20	53.7	51.6	57.6	1.0	1.3	3.7
23	46.0	18.4	20.4	7.5	8.0	3.3
26	57.3	44.1	41.8	6.0	4.7	1.7
27	54.4	50.0	46.6	6.4	0.1	7.6
29	12.5	22.8	26.5	1.1	10.1	8.3
35	2.4	2.8	1.4	0.3	0.9	0.2
36	12.6	13.1	7.7	4.9	6.6	7.2
37	36.0	33.8	27.3	5.6	4.6	2.9
38	33.1	35.1	30.6	4.0	5.2	3.2
39	23.9	26.6	26.9	2.0	2.7	2.3
40	38.2	19.1	33.9	6.1	0.7	6.1
41	38.7	26.1	39.4	5.1	1.7	4.6
42	33.3	17.1	29.4	5.1	2.2	2.9
45	24.4	25.6	30.0	5.9	0.3	3.5
46	27.8	30.4	34.3	4.7	4.9	5.7
47	30.0	33.1	29.7	4.7	2.8	2.2
48	46.0	56.0	46.8	5.1	5.9	7.8
55	20.1	23.0	24.9	2.0	3.8	1.7
56	33.0	38.5	44.1	6.7	6.6	0.2
57	46.4	47.8	52.1	7.3	3.0	4.8
59	42.5	40.4	49.3	7.7	5.6	5.6
66	41.0	40.5	32.1	9.8	2.4	21.0
72	30.3	13.1	33.7	1.9	7.3	0.1
73	31.5	16.9	41.9	4.0	6.0	6.5
74	27.7	12.4	22.6	1.0	5.3	1.5
75	37.2	21.0	35.2	4.8	7.2	0.0
76	10.1	14.4	8.7	1.9	3.8	0.7
78	33.0	37.1	28.9	6.5	2.5	7.3

Table 4-46: Shows for each SMU recorded in the slow ramp jaw closing task, the mean thresholds of all repetitions for each block and the standard deviation.

For the slow ramp task (n=37), 17 SMUs had a higher mean of threshold for hypertonic when comparing with isotonic, while 20 SMUs exhibited a decrease in threshold mean for the hypertonic saline block when compared with the isotonic saline block.

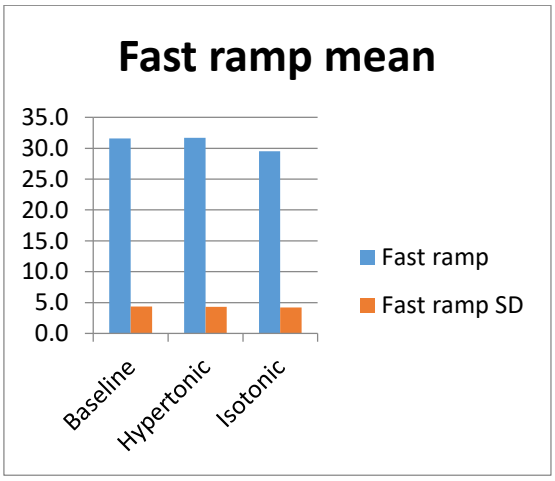


Figure 4-19: Mean and SD (N) for threshold of onset of SMU activity in the fast ramp jaw closing task.

For the fast ramp task it is possible to notice that the isotonic threshold overall mean was lower compared with the other blocks, however, the main effect of session (baseline, hypertonic infusion, isotonic infusion) was not statistically different ($p>0.05$) across all threshold values for the slow ramp tasks.

A further analysis was done for each unit individually in order to determine if, even with no change in the overall activity, some units would possibly show an effect on their individual threshold values. This individual SMU analysis is shown in Table 4-47.

SMU	Baseline	Hypertonic	Isotonic	B SD	H SD	I SD

7	38.8	40.6	24.2	1.6	6.7	7.6
11	4.5	19.9	4.5	2.4	7.1	2.8
12	27.4	31.7	22.6	7.0	2.8	6.1
13	51.4	64.5	43.9	7.1	2.0	0.7
14	5.7	13.2	5.3	4.2	13.5	3.7
15	44.2	46.6	30.9	6.6	11.8	6.1
16	21.5	29.0	31.9	1.7	4.7	4.4
17	48.6	46.2	55.2	0.2	3.8	5.5
19	59.1	53.0	55.3	4.0	2.2	3.3
23	46.9	23.0	24.9	3.6	3.1	6.8
26	53.6	47.3	43.0	3.4	11.8	7.3
29	7.8	17.1	30.7	0.8	0.4	2.4
35	2.3	2.9	2.6	0.4	0.5	1.4
36	7.8	18.5	9.9	3.2	2.3	2.9
37	30.1	33.6	18.2	5.9	7.5	5.9
38	36.4	33.6	24.1	0.7	5.4	4.6
39	22.0	29.9	35.9	2.8	7.6	6.4
40	32.0	24.1	35.7	7.5	0.6	2.1
42	32.9	24.5	34.4	2.8	2.1	1.5
45	27.7	28.6	23.6	5.8	5.7	2.7
46	35.2	29.5	25.9	6.1	7.8	3.6
47	32.9	26.4	25.8	5.2	6.5	4.9
55	26.6	31.0	26.6	6.2	0.5	5.2
56	42.0	32.0	42.5	4.7	5.3	0.9
57	49.8	63.9	49.2	1.7	7.4	4.9
59	52.0	63.2	47.1	9.1	1.6	5.6
66	58.3	35.2	49.7	0.0	6.5	6.4
72	16.4	16.9	29.3	1.9	4.5	3.6
73	29.8	21.9	39.1	3.2	5.3	4.4
74	19.2	15.1	17.8	6.6	0.8	2.4
75	34.4	25.9	38.2	4.8	0.8	2.4
76	9.1	22.6	8.7	3.8	4.7	3.7
77	21.4	20.7	15.0	6.1	3.1	3.6
78	35.9	44.0	32.3	1.6	2.0	1.3
80	42.4	33.7	28.9	2.7	2.6	1.1

Table 4-47: Shows for each SMU recorded in the slow ramp jaw closing task, the means of repetitions for each block and the standard deviation.

For the fast ramp task (n= 35), 21 units had a higher mean of threshold for hypertonic when comparing with isotonic, where 14 units exhibited a decrease in threshold mean for the painful block when compared with the isotonic block.

4.4.3.6 Firing rates for step tasks

The data of 62 discriminated SMUs were further analyzed to calculate the firing rates, that is, how many times that unit was active per second, during the 2 s of stable period of the step 1 and step 2 of the 2 step-levels jaw closing task. From all the units analyzed, only those units that could be clearly discriminated are presented in the following analysis. The firing rates of some SMUs are not presented as it was not possible to confidently discriminate these SMUs throughout the entire stable period.

The comparison in this thesis is on the difference between the hypertonic block and the isotonic block. Therefore, it was possible to study the activity of 20 SMUs under both these blocks during the step 1 and 15 SMUs during the step 2.

Table 4-48 lists the firing rates of the 20 SMUs characterized during the step 1 of the 2 step-levels jaw closing task.

Step 1:

Participant	SMU	Hyper	Iso	Higher firing rate	Solution inserted first
1	1	17.7	22.7	Isotonic	Hypertonic
	2	18.8	19.2	Isotonic	
	3	-	22.9	Isotonic	
	5	16.2	-	Hypertonic	
2	7	16.0	21.1	Isotonic	Isotonic
5	16	8.8	6.1	Hypertonic	Hypertonic
6	26	20.1	15.5	Hypertonic	Isotonic
	27	-	5.3	Isotonic	
7	29	15.9	15.8	Hypertonic	Hypertonic
	30	10.0	11.7	Isotonic	
11	35	14.0	15.8	Isotonic	Hypertonic
	36	19.5	18.1	Hypertonic	
13	45	9.5	10.9	Isotonic	Hypertonic
	46	11.0	9.3	Hypertonic	
	49	12.0	9.0	Hypertonic	
15	55	15.1	19.5	Isotonic	Hypertonic
16	60	11.0	15.0	Isotonic	Hypertonic
	63	-	19.7	Isotonic	
17	69	16.0	-	Hypertonic	Isotonic
19	76	-	8.7	Isotonic	Isotonic

Table 4-48: Firing rates of SMUs analysed for step 1 of the 2 step-levels jaw closing task. In grey is shown the firing rates for units that were present in only one of the two blocks. Yellow highlight for hypertonic saline block: firing rate was lower than the isotonic saline block; Blue highlight: firing rate was higher. Hyper: hypertonic saline infusion block; Iso: isotonic saline infusion block.

A qualitative analysis was carried out and of these 20 SMUs, 8 SMUs “1, 2, 7, 30, 35, 45, 55, 60” showed an decrease in firing rates when comparing hypertonic vs. isotonic recording sessions, and 6 SMUs “16, 26, 29, 36, 46, 49” showed an increase in firing rates. Also, 4 single motor units “3, 27, 63, 76” were absent during the hypertonic session while 2 single motor units “5, 69” were absent for the isotonic session. There did not appear to be any association between whether there was a decrease in firing

rate or an increase in firing rate and the sequence of infusion, that is, whether hypertonic saline infusion was performed first, or isotonic saline infusion was performed first.

Table 4-49 lists the firing rates of the 15 SMUs characterized during the step 2 of the 2 step-levels jaw closing task.

Step 2:

Participant	SMU	Hyper	Iso	Higher firing rate	Solution inserted first
1	1	22.2	10.0	Hypertonic	Hypertonic
	2	19.0	11.0	Hypertonic	
	3	-	12.0	Isotonic	
	4	-	15.9	Isotonic	
	5	19.1	-	Hypertonic	
4	13	13.5	11.4	Hypertonic	Isotonic
10	34	18.0	18.1	Isotonic	Isotonic
11	37	22.0	20.3	Hypertonic	Hypertonic
	38	22.5	16.3	Hypertonic	
13	51	15.0	8.0	Hypertonic	Hypertonic
15	55	23.3	21.5	Hypertonic	Hypertonic
	56	18.3	17.3	Hypertonic	
	57	14.0	17.3	Isotonic	
	59	23.0	18.7	Hypertonic	
19	76	15.1	17.3	Isotonic	Isotonic

Table 4-49: Firing rates of SMUs analysed for step 2 of the 2 step-levels jaw closing task. In grey is shown the firing rates for units that were present in only one of the two blocks. Yellow highlight for hypertonic saline block: firing rate was lower than the isotonic saline block; Blue highlight: firing rate was higher. Hyper: hypertonic saline infusion block; Iso: isotonic saline infusion block.

A qualitative analysis was carried out and of these 15 SMUs, 3 single motor units “34, 57, 76” showed a decrease in firing rates, 9 single motor units “1, 2, 13, 37, 38, 51, 55, 56, 59” showed an increase in firing rates when comparing hypertonic vs. isotonic

recording sessions. Also, 2 single motor units “3, 4” suffered a de-recruitment for hypertonic session while 1 single motor units “5” suffered a de-recruitment for the isotonic session.

A repeated measures analysis of variance (ANOVA) of all discriminated SMU firing rates values for each jaw task across all of the blocks was performed to determine the main effect of repeating the jaw task and of the infusing saline into the masseter muscle.

The results showed no significant effect on the firing values of repeating the task during the baseline, hypertonic saline infusion, or isotonic saline infusion of the 2 step-levels jaw closing task.

The overall mean of the firing rates for all the SMUs was then calculated for each block in order to determine if there was an effect of block on SMU firing rates for the 2 step-levels jaw closing tasks and no significant difference was found for either of the levels ($p>0.05$).

No difference between repetitions:

Step 1:

Baseline: $p=0.652$ ($n = 11$)

Hypertonic $p=0.643$ ($n = 9$)

Isotonic p=0.411 (n = 14)

Step 2:

Baseline: p=0.405 (n = 6)

Hyper p=0.058 (n = 5)

Isotonic p=0.89 (n=6)

Multiple comparisons showed no significant difference between blocks in firing rates

Step 1 between blocks: p=0.950 (n= 13)

Step 2 between blocks: p=0.215 (n =8)

4.4.3.7 Sequence of recruitment for each block

The data was further analysed to determine whether the sequence of recruitment of the SMUs was altered when comparing baseline, hypertonic saline infusion and isotonic saline infusion blocks. Units were only considered if they were present in all 3 of the baseline, hypertonic saline infusion and isotonic saline infusion blocks. Participants that only had one unit that was present in the three blocks were also not considered in this analysis as it is not possible to provide a recruitment sequence.

The data from 12 participants were able to be analysed for this recruitment analysis for the slow ramp jaw closing task based on the mean thresholds and this analysis is shown on Table 4-50.

ID	Baseline SR	Hypertonic SR	Isotonic SR	BxH	BxI	HxI	First solution
1	1 / 2	2 / 1	2 / 1	Different	Different	Same	H
2	7 / 8	7 / 8	7 / 8	Same	Same	Same	I
4	14 / 12 / 11 / 15 / 13	14 / 11 / 12 / 15 / 13	14 / 11 / 12 / 15 / 13	Different	Different	Same	I
5	16 / 17 / 19 / 20	16 / 19 / 17 / 20	16 / 17 / 19 / 20	Different	Same	Different	H
6	23 / 26 / 27	23 / 26 / 27	23 / 26 / 27	Same	Same	Same	I
11	35 / 36 / 39 / 38 / 37	35 / 36 / 39 / 37 / 38	35 / 36 / 37 / 39 / 38	Different	Different	Different	H
12	42 / 40 / 41	42 / 40 / 41	42 / 40 / 41	Same	Same	Same	I
13	45 / 46 / 47 / 48	45 / 46 / 47 / 48	45 / 47 / 46 / 48	Same	Different	Different	H
15	55 / 56 / 57 / 59	55 / 56 / 59 / 57	55 / 56 / 59 / 57	Different	Different	Same	H
16	60 / 61 / 63	61 / 60 / 63	60 / 61 / 63	Different	Same	Different	H
18	74 / 72 / 73 / 75	74 / 72 / 73 / 75	74 / 72 / 73 / 75	Same	Same	Same	H
19	76 / 77 / 78	76 / 77 / 78	76 / 77 / 78	Same	Same	Same	I

Table 4-50 : Sequence of recruitment of SMUs in the temporalis muscle during the slow ramp jaw closing task (SR). Each SMU is indicated by a number and the order in which each SMU is recruited is indicated by the sequence of those numbers. The comparison between blocks is shown as “different” when at least one unit appeared in a different order in one of those blocks, and “same” when all the units appeared in the exactly the same order in both of those blocks. In order to analyse a possible post pain effect, the infusion injected first is also mentioned on the table. SR: slow ramp jaw closing task; B: baseline; I: isotonic saline infusion; H: hypertonic saline infusion.

For the slow ramp jaw closing task, 6 participants had a different order when comparing the baseline with the hypertonic saline block, 5 participants had a different sequence of recruitment when comparing the baseline with the isotonic saline block and 4 participants had a different sequence when comparing the hypertonic saline infusion block with the isotonic saline infusion block.

The data from 12 participants were able to be analysed for this recruitment analysis for the fast ramp jaw closing task based on the mean thresholds and this analysis is shown on Table 4-51.

ID	Baseline FR	Hypertonic FR	Isotonic FR	BxH	BxI	HxI	First solution
1	1/2	2 / 1	1 / 2	Different	Same	Different	H
4	11 / 14 / 12 /15 / 13	11 / 14 / 12 /15 / 13	11 / 14 / 12 /15 / 13	Same	Same	Same	I
5	16 / 17 / 19	16 / 17 / 19	16 / 19 / 17	Same	Different	Different	H
6	23 / 27 / 26	23 / 26 / 27	23 / 26 / 27	Different	Different	Same	I
7	29 / 30	29 / 30	29 / 30	Same	Same	Same	H
11	35 / 36 / 39 / 37 / 38	35 / 36 / 38 / 39 / 37	35 / 36 / 37 / 38 / 39	Different	Different	Different	H
12	42 / 40	40 / 42	42 / 40	Different	Same	Different	I
13	45 / 46 / 47	45 / 46 / 47	45 / 47/ 46	Same	Different	Different	H
15	55 / 56 / 57 / 59	55 / 56 / 59 / 57	55 / 56 / 59 / 57	Different	Different	Same	H
16	60 / 63	60 / 63	60 / 63	Same	Same	Same	H
18	74 / 72 / 73 / 75	74 / 72 / 73 / 75	74 / 72 / 73 / 75	Same	Same	Same	H
19	76 / 77 / 78 / 80	77 / 76 / 80 / 78	77 / 76 / 80 / 78	Different	Different	Same	I

Table 4-51**:** Sequence of recruitment of SMUs in the temporalis muscle during the fast ramp jaw closing task (FR). Each SMU is indicated by a number and the order in which each SMU is recruited is indicated by the sequence of those numbers. The comparison between blocks is shown as “different” when at least one unit appeared in a different order in one of those blocks, and “same” when all the units appeared in the exactly the same order in both of those blocks. In order to analyse a possible post pain effect, the infusion inserted first was also mentioned on the table. FR: fast ramp jaw closing task; B: baseline; I: isotonic saline infusion; H: hypertonic saline infusion.

For the fast ramp jaw closing task, it is possible to notice that 6 participants had a different order comparing baseline with hypertonic block, 6 participants had a different sequence of recruitment when comparing baseline with isotonic block and 5 participants had a different sequence between hypertonic and isotonic.

4.4.4 Occurrence of single motor units in the right masseter muscle:

In total, 58 SMUs were discriminated from the masseter muscle from 15 participants. Among those 58 SMUs, 50 units were discriminated in at least one of the ramp jaw closing tasks, while 47 (39 also present in the ramps + 8 new SMUs) were discriminated for at least one step level (step 1 and/or step 2) of the 2 step-levels jaw closing task – See figure 4-20.

8 units (5, 6, 7, 8, 12, 17, 32, 33) were present exclusively for the step tasks, while 6 units (1, 2, 3, 4, 30, 31) were present in the ramp jaw closing tasks but it was not possible to discriminate from other units in the step tasks. Units 4, 30 and 43 were present in the fast ramp jaw closing task but not in the slow ramp jaw closing task and the unit 39 was present in the slow ramp jaw closing task but not in the fast ramp jaw closing task.

For an easier understanding, the units presented for ramps jaw closing tasks (slow and fast) and the units presented for the 2 step-levels jaw closing task will be described separately on the following sections (section 4.4.4.1 and section 4.4.4.2, respectively).

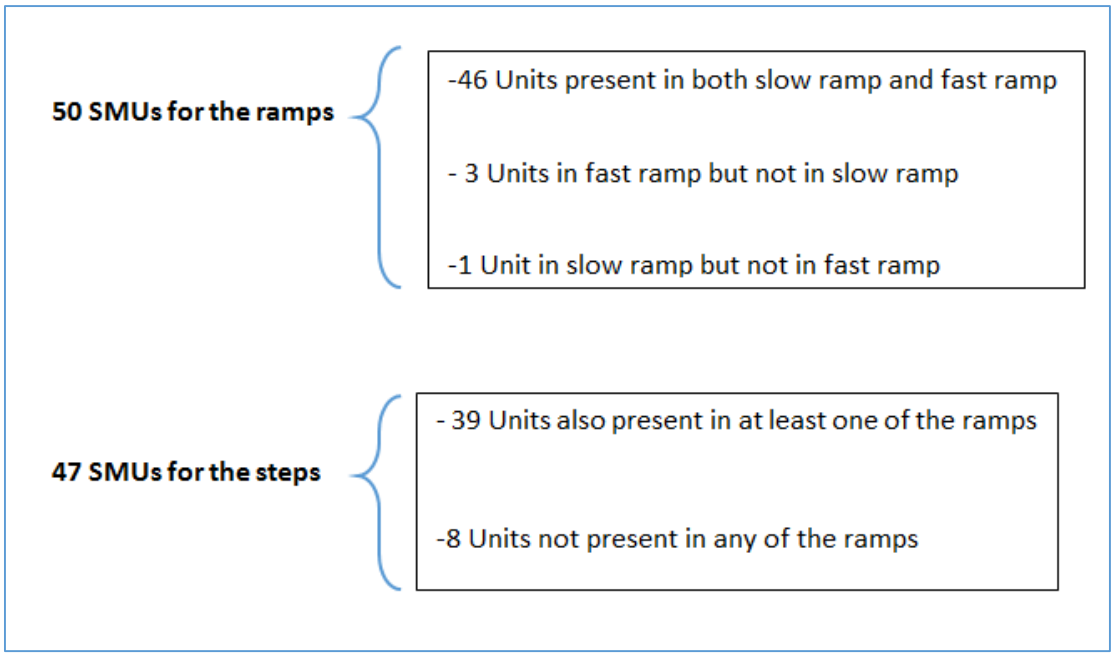


Figure 4-20: Summary of units (i.e. SMUs) in the tasks for the right masseter muscle. Ramps = slow ramp and fast ramp jaw closing tasks; Steps = Step 1 of the 2 step-levels jaw closing task + Step 2 of the 2 step-levels jaw closing task.

4.4.4.1 Occurrence of motor units in slow ramp and fast ramp tasks for the masseter muscle

Table 4-52 lists the occurrences of the 50 SMUs in each recording block (baseline, hypertonic saline infusion block, isotonic saline infusion block) for the ramp tasks (slow and fast ramp jaw closing tasks) in the masseter muscle. If a SMU was present in at least 2 of the 3 trials done or at least half of trials when more than 3 trials were done, then that SMU was marked as "+" (i.e. present). If not, that SMU was marked as "-" (i.e. not present).

		Slow ramp			Fast Ramp		
Participant	SMU	BS	Hyper	Iso	BS	Hyper	Iso
	1	+	+	+	+	-	+
	2	+	-	+	+	-	+
	3	+	+	+	+	+	+
5	4	-	-	-	-	+	-
	9	+	+	+	+	+	+
	10	+	+	+	+	+	+
6	11	-	+	-	-	+	+
	13	+	+	+	+	+	+
	14	+	+	+	+	+	+
	15	+	+	+	+	+	+
7	16	-	+	-	-	+	-
	18	+	+	+	+	+	+
8	19	+	+	+	+	+	+
	20	+	+	+	+	+	+
	21	+	+	+	+	+	+
9	22	+	+	+	+	+	+
	23	+	+	+	+	+	+
10	24	+	+	+	+	+	+
	25	+	+	+	+	+	+
	26	+	+	+	+	+	-
11	27	-	+	+	-	+	+
	28	+	+	+	+	+	+
	29	+	+	+	-	+	+
	30	-	-	-	+	-	-
12	31	+	+	+	+	+	+
	34	+	-	-	+	-	-
	35	+	-	-	+	-	-
13	36	-	+	+	+	+	+
	37	+	+	+	+	+	+
	38	+	-	+	+	-	+
	39	+	-	-	-	-	-
	40	-	+	+	+	+	+
	41	-	+	-	-	+	-
	42	-	+	-	+	+	+
14	43	-	-	-	+	-	-
16	44	+	+	+	+	+	+

		Slow ramp			Fast Ramp		
Participant	SMU	BS	Hyper	Iso	BS	Hyper	Iso
	45	+	+	+	+	+	+
	46	+	-	+	+	-	+
17	47	-	-	+	-	+	+
	48	+	-	+	+	-	+
	49	+	-	+	-	-	+
	50	-	+	-	-	+	-
18	51	-	+	-	-	+	-
	52	+	+	+	+	+	+
	53	+	+	+	+	+	+
	54	+	+	+	+	+	+
19	55	+	-	+	+	+	+
	56	+	+	+	+	+	+
	57	+	+	+	+	+	+
20	58	-	-	+	+	+	+

Table 4-52: All the SMUs (n=50) present in the slow ramp and fast ramp jaw closing tasks under each block. Highlighted in grey are the units that were present in one speed of the slow or fast ramp jaw closing task but which were not present in the other. BS: baseline; hyper: hypertonic saline infusion block; iso: isotonic saline infusion block.

Among the 50 units present for the ramp jaw closing tasks, 24 SMUs did not change their pattern of occurrence and were present in both fast and slow ramp jaw closing tasks for baseline, hypertonic saline infusion and isotonic saline infusion blocks. Table 4-53 shows those 24 SMUs that were present in all the tasks for the 3 blocks.

In addition, 4 participants (Participant 8, 9, 10 and 16) showed no change in the pattern of recruitment of all the units discriminated from the masseter muscle in each of those participants. Therefore, these participants presented the same units (SMUs 18, 19 – for participant 8; SMUs 20, 21, 22 - for participant 9; SMUs 23, 24 - for participant 10; and SMUs 44 for participant 16) for both tasks (slow ramp and fast ramp jaw closing

tasks) during the three blocks analysed (baseline, hypertonic saline infusion, isotonic saline infusion; see Table 4-53 highlighted).

		Slow ramp			Fast Ramp		
Participant	SMU	BS	Hyper	Iso	BS	Hyper	Iso
5	3	+	+	+	+	+	+
	9	+	+	+	+	+	+
6	10	+	+	+	+	+	+
	13	+	+	+	+	+	+
	14	+	+	+	+	+	+
7	15	+	+	+	+	+	+
	18	+	+	+	+	+	+
8	19	+	+	+	+	+	+
	20	+	+	+	+	+	+
	21	+	+	+	+	+	+
9	22	+	+	+	+	+	+
	23	+	+	+	+	+	+
10	24	+	+	+	+	+	+
11	25	+	+	+	+	+	+
	28	+	+	+	+	+	+
12	31	+	+	+	+	+	+
14	37	+	+	+	+	+	+
16	44	+	+	+	+	+	+
17	45	+	+	+	+	+	+
	52	+	+	+	+	+	+
	53	+	+	+	+	+	+
19	54	+	+	+	+	+	+
	56	+	+	+	+	+	+
20	57	+	+	+	+	+	+

Table 4-53: SMUs (n=24) that were present in all the tasks for the baseline, hypertonic saline infusion and isotonic saline infusion block. In grey, participants who had no change in the pattern of occurrence for all the units discriminated from each of those participants. BS: baseline; hyper: hypertonic saline infusion block; iso: isotonic saline infusion block.

Of the 50 SMUs, the remaining 26 exhibited some change in the pattern of SMU occurrence between the blocks (n=10), or between the blocks and between the slow and fast ramp jaw closing tasks (n=16) – See Table 4-54 and 4-55.

From those 26 SMUs, 10 units (2, 16, 27, 34, 35, 38, 41, 46, 48, 50) showed the same pattern of occurrence for the slow ramp jaw closing tasks and the fast ramp jaw closing tasks, and the change in occurrence was only due to the block (yellow highlighted SMUs in Table 4-54). The remaining 16 units (1, 4, 11, 26, 29, 30, 36, 39, 40, 42, 43, 47, 49, 51, 55, 58) had different patterns of occurrence for the different tasks as well as changes in occurrence between the blocks. One unit (39) was found in the slow ramp jaw closing task, but not in the fast ramp jaw closing task, while 3 units (4, 30, 43,) were found for fast ramp jaw closing task but not for the slow ramp jaw closing task.

		Slow ramp			Fast Ramp		
Participant	SMU	BS	Hyper	Iso	BS	Hyper	Iso
	1	+	+	+	+	-	+
	2	+	-	+	+	-	+
	4	-	-	-	-	+	-
	11	-	+	-	-	+	+
5	16	-	+	-	-	+	-
11	26	+	+	+	+	+	-
	27	-	+	+	-	+	+
12	29	+	+	+	-	+	+
	30	-	-	-	+	-	-
	34	+	-	-	+	-	-
	35	+	-	-	+	-	-
13	36	-	+	+	+	+	+
	38	+	-	+	+	-	+
	39	+	-	-	-	-	-
	40	-	+	+	+	+	+
14	41	-	+	-	-	+	-
	42	-	+	-	+	+	+
	43	-	-	-	+	-	-
17	46	+	-	+	+	-	+
	47	-	-	+	-	+	+
	48	+	-	+	+	-	+
	49	+	-	+	-	-	+
	50	-	+	-	-	+	-
18	51	-	+	-	-	+	-
19	55	+	-	+	+	+	+
20	58	-	-	+	+	+	+

Table 4-54: Shows only those SMUs (n=26) that exhibited some change in the pattern of occurrence between blocks (n=10; yellow highlighted SMUs), or between the blocks and between the slow and fast ramp jaw closing tasks (n=16). BS: baseline; hyper: hypertonic saline infusion block; iso: isotonic saline infusion block.

		Slow ramp			Fast Ramp		
Participant	SMU	BS	Hyper	Iso	BS	Hyper	Iso
5	2	+	-	+	+	-	+
7	16	-	+	-	-	+	-
11	27	-	+	+	-	+	+
13	34	+	-	-	+	-	-
	35	+	-	-	+	-	-
14	38	+	-	+	+	-	+
	41	-	+	-	-	+	-
17	46	+	-	+	+	-	+
18	48	+	-	+	+	-	+
	50	-	+	-	-	+	-

Table 4-55: Shows only those units (n=10) that did not exhibit a change in the pattern of occurrence across all 3 blocks between the slow ramp and fast ramp jaw closing tasks. BS: baseline; hyper: hypertonic saline infusion block; iso: isotonic saline infusion block.

According to the tables above (4-54 and 4-55), it is possible to notice that some units were present or were not present within the hypertonic saline infusion block. Units 11, 16, 41, 50, 51, for example, were present only during hypertonic saline infusion blocks for slow and fast ramps jaw closing tasks but were not present during baselines or isotonic saline infusion blocks. Units 2, 38, 46, 48, 55, on the other hand, were not present for slow and fast ramps jaw closing tasks during hypertonic saline infusion block but were present during baselines and isotonic saline infusion blocks for slow and fast ramps jaw closing tasks.

Units 34 and 35 were not present in hypertonic and isotonic saline infusion but were present for the baseline block; while unit 27 was present in hypertonic saline infusion and isotonic saline infusion block but not present for the baseline block.

For all the other participants (Participant 5, 11, 12, 13, 14, 17, 18, 19, 20), it was possible to note some change in the pattern of occurrence of units not only within a block, but also with different patterns between slow ramp and fast ramp jaw closing tasks (n=16) – See table 4-56.

		Slow ramp			Fast Ramp		
Participant	SMU	BS	Hyper	Iso	BS	Hyper	Iso
	1	+	+	+	+	-	+
	4	-	-	-	-	+	-
5 (H)	11	-	+	-	-	+	+
11 (H)	26	+	+	+	+	+	-
12 (I)	29	+	+	+	-	+	+
	30	-	-	-	+	-	-
13 (H)	36	-	+	+	+	+	+
14 (I)	39	+	-	-	-	-	-
	40	-	+	+	+	+	+
	42	-	+	-	+	+	+
	43	-	-	-	+	-	-
17 (I)	47	-	-	+	-	+	+
18 (H)	49	+	-	+	-	-	+
	51	-	+	-	-	+	-
19 (I)	55	+	-	+	+	+	+
20 (H)	58	-	-	+	+	+	+

Table 4-56: Shows only those units (n=16) that exhibited a change in the pattern of occurrence between slow ramp and fast ramp. Letter in brackets indicates the solution applied first for that participant and is noted after the participant's number as (H) for hypertonic saline being applied first and (I) when the isotonic solution was applied first. BS: baseline; hyper: hypertonic saline infusion block; iso: isotonic saline infusion block.

Unit 5 was present only during hypertonic saline infusion fast ramp jaw closing task but was not present in the other blocks for slow ramp jaw closing task. Unit 1, on the other hand, was not present during hypertonic saline infusion fast ramp jaw closing task but

it was present in the slow ramp jaw closing tasks (all the blocks) and for the remaining blocks in the fast ramp jaw closing tasks.

Figure 4-21 Summarizes schematically the 50 SMUs discriminated for the ramps jaw closing tasks in this study.

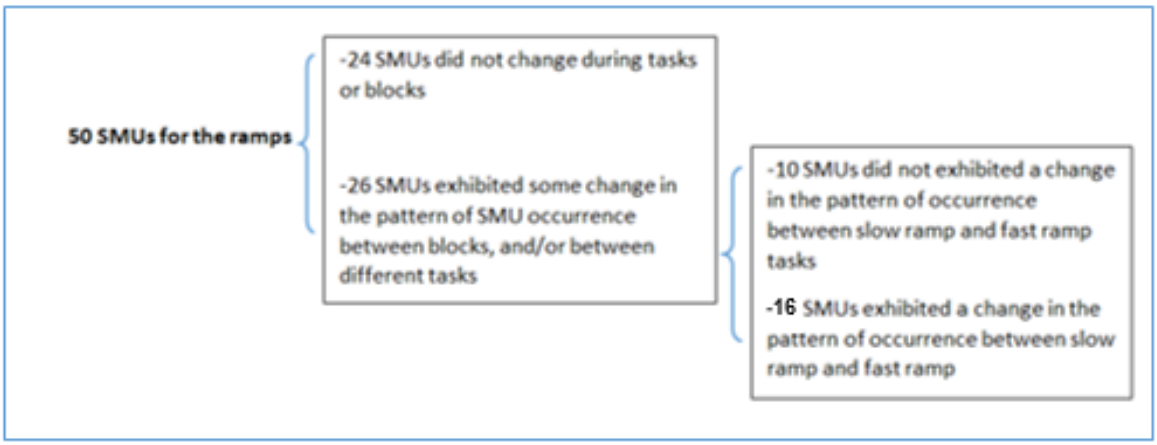


Figure 4-21: Schematic figure to summarize the 50 SMUs discriminated for the ramps tasks in the right masseter muscle in this study. Unit = SMU.

16

4.4.4.2 *Occurrence of motor units in steps for masseter muscle:*

The 47 motor units present in the 2 step-levels jaw closing tasks are described in Table 4-57. Of the 47 SMUs, 39 were also present in one or more of the ramp jaw closing tasks and the remaining 8 were new SMUs that were only present in the 2 step-levels jaw closing task.

		Step 1			Step 2		
Participant	SMU	BS	Hyper	Iso	BS	Hyper	Iso
5	5	+	-	+	-	-	-
	6	-	+	-	+	+	+
	7	-	-	-	+	+	+
	8	-	-	-	+	+	+
6	9	+	+	+	?	?	?
	10	+	+	+	?	?	?
	11	-	-	-	+	+	+
	12	-	-	-	+	+	+
7	13	+	+	+	?	?	?
	14	+	+	+	?	?	?
	15	-	-	-	?	?	?
	16	-	+	+	?	?	?
	17	-	-	-	+	+	+
8	18	+	+	+	+	+	+
	19	+	+	+	+	+	+
9	20	+	+	+	+	+	+
	21	-	-	+	+	+	+
	22	-	+	+	+	+	+
10	23	+	+	+	+	+	+
	24	+	+	+	+	+	+
11	25	-	+	-	+	+	+
	26	-	-	-	-	+	+
	27	+	+	+	+	+	+
12	28	+	+	+	+	+	+
	29	+	+	+	+	+	+
	32	+	+	+	?	?	?
	33	-	-	-	-	+	+

Participant		Step 1			Step 2		
	SMU	BS	Hyper	Iso	BS	Hyper	Iso
13	34	+	-	-	+	-	+
	35	+	-	-	+	-	-
	36	+	-	+	+	-	+
	37	+	+	+	+	+	+
	38	+	+	+	+	+	+
	39	-	-	-	?	?	?
	40	-	-	-	+	+	+
	41	-	-	-	?	?	?
	42	-	-	-	+	+	+
14	43	-	-	-	?	?	?
	45	-	-	-	+	+	+
	46	-	-	-	+	+	+
17	47	-	-	-	+	+	+
	48	?	?	?	?	?	?
	49	?	?	?	?	?	?
	50	?	?	?	?	?	?
18	51	?	?	?	?	?	?
	52	+	+	+	+	+	+
	53	+	+	+	+	+	+
	54	-	-	-	+	+	+
19	55	-	+	-	?	?	?
	56	+	+	+	+	+	+
	57	+	+	+	+	+	+
20	58	-	-	-	+	+	+

Table 4-57: Motor units (n=47) discriminated in the 2 step-levels jaw closing tasks. In grey, all the motor units that were exclusively found for the steps tasks and were not present in the ramp tasks. ? = when it was not possible to confirm the presence or absence of the unit for that task. BS: baseline; hyper: hypertonic saline infusion block; iso: isotonic saline infusion block.

From the 47 units described in the Table 4-57, 29 units were discriminated for step 1 and 35 units were discriminated for step 2; these 35 were made up of 21 present for step 1 plus and an additional 14 newly recruited SMUs. Due to the greater force and higher level of EMG activity for step 2, it was not possible to confirm precisely if 4 small

units (48, 49, 50, 51) were present or absent in the step 1 and if 14 units (9, 10, 13, 14, 15, 16, 32, 39, 41, 43, 48, 49, 50, 51) were present or absent in step 2. For those cases, a question mark was used in the table.

For the step 1 of the 2 step-levels jaw closing task, from the 29 units discriminated, 19 units (9, 10, 13, 14, 18, 19, 20, 23, 24, 27, 29, 32, 37, 38, 52, 53, 57) were present in the three blocks, 3 units (6, 25, 55) were recruited only for the hypertonic block, 2 units (5, 36) were de-recruited only for the hypertonic block and the remaining 5 units (16, 21, 22, 34, 35) were not present in some of the blocks.

For the step 2 of the 2 step-levels jaw closing task, from the 35 units discriminated, 30 units (6, 7, 8, 11, 12, 17, 18, 19, 20, 21, 22, 23, 24,25, 27, 28, 29, 37, 38, 40, 42, 45, 46, 47, 52, 53, 54, 56, 57, 58) were present in the three blocks, 2 units (34 and 36) were de-recruited only for the hypertonic block, 1 unit (35) was recruited only for the baseline but was not present in the hypertonic or isotonic blocks, and 2 units (26 and 33) were not present in the baseline block but were present for both blocks of infusion (hypertonic and isotonic).

Figure 4-22 Summarizes schematically the 47 SMUs discriminated for the 2 step-levels jaw closing tasks in this study.

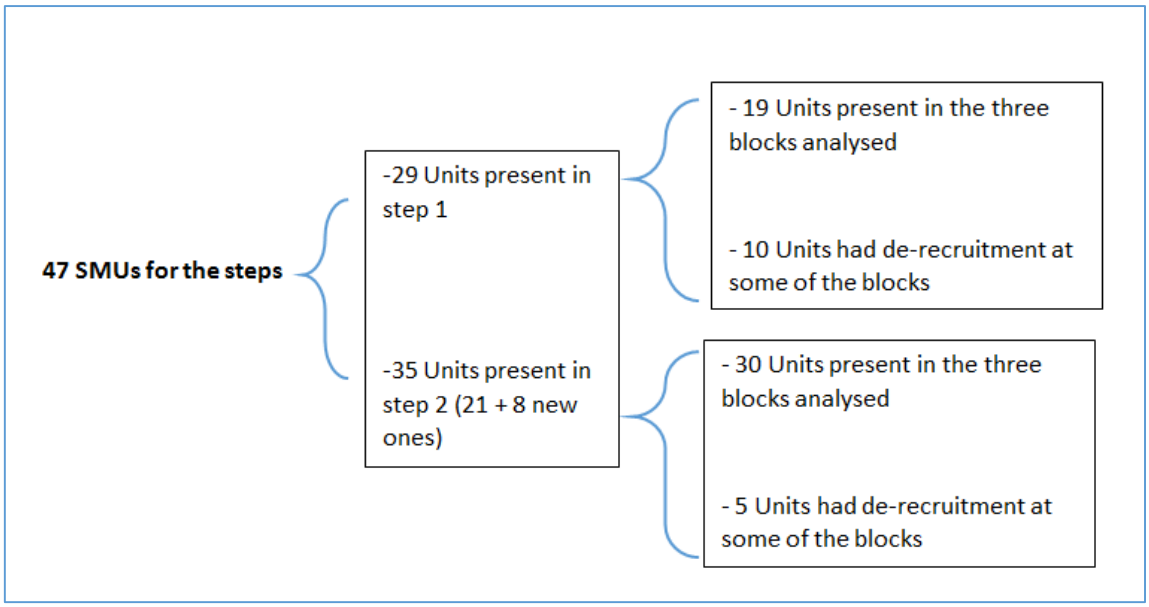


Figure 4-22: Schematic figure to summarize the 47 SMUs discriminated for the steps tasks in the right masseter muscle in this study. Unit = SMU.

4.4.4.3 Comparisons between blocks:

For each of the slow ramp and fast ramp jaw closing tasks, and step 1 and step 2 of the 2 steps-levels jaw closing task, the hypertonic saline infusion block (H) was compared with all the other blocks - baseline (B) and Isotonic saline infusion (I) - in terms of the number of units during the hypertonic saline infusion block of tasks that

- Exhibited no change in the pattern of occurrence between the 2 blocks,
- Became present in the hypertonic saline infusion block (i.e. were recruited), or
- Was not present (i.e. was de-recruited) during the hypertonic saline infusion block.

These analyses are shown in Tables 4-58 and 4-59.

	B x H SR	B x H FR	B x H step 1	B x H step 2
No change	29/47 (61.7%)	30/49 (61.2%)	17/28 (60.7%)	30/35 (85.7%)
Recruited	9/47 (19.1%)	8/49 (16.3%)	4/28 (14.3%)	2/35 (5.7%)
De-recruited	9/47 (19.1%)	11/49 (22.4%)	7/28 (25%)	3/35 (8.6%)

Table 4-58: Describes the comparison between the hypertonic saline infusion block with the baseline block for slow ramp, fast ramp, step 1 and step 2 jaw closing tasks. For the slow ramp tasks, 61.7% of the units did not change, 61.2% of the units did not change for the fast ramp, and 60.7% and 85.7% of the units did not change for step 1 and step 2 respectively. Recruited = SMU became present in the hypertonic saline infusion block; de-recruited = were not present during the hypertonic saline infusion block

	I x H SR	I x H FR	I x H step 1	I x H step 2
No change	33/47 (70.2%)	37/49 (75.6%)	23/28 (82.1%)	33/35 (94.3%)
Recruited	6/47 (12.8%)	6/49 (12.2%)	2/28 (7.1%)	0/35 (0%)
De-recruited	8/47 (17%)	6/49 (12.2%)	3/28 (10.8%)	2/35 (5.7%)

Table 4-59: Describes the comparison between the hypertonic saline infusion block with the isotonic saline infusion block for slow ramp, fast ramp, step 1 and step 2 jaw closing tasks. For the slow ramp tasks, 70.2% of the units did not change, 75.6% of the units did not change for the fast ramp, and 82.1% and 94.3% of the units did not change for step 1 and step 2 respectively. Recruited = SMU became present in the hypertonic saline infusion block; de-recruited = were not present during the hypertonic saline infusion block

4.4.4.4 *Masseter SMU activity and the vicious cycle theory and the pain adaptation model*

An analysis was performed to determine whether the change in the pattern of occurrence of SMUs recorded in the masseter muscle could be explained on the basis of the principles outlined for the Vicious Cycle Theory or the Pain Adaptation Model. This analysis is summarized in Table 4-60.

If the SMU was recruited during the hypertonic saline infusion block but was inactive (i.e. not present) in the isotonic saline infusion block, then the pattern of occurrence of the SMU was considered to be consistent with the Vicious Cycle Theory as the pain, according to this theory, would cause “muscle hyperactivity”. On the other hand, if the SMU becomes inactive (i.e. not present) during the hypertonic saline infusion block in comparison with the isotonic saline infusion block, this pattern of occurrence was considered to be consistent with the Pain Adaptation Model which proposes decreased agonist muscle activity in pain so as to result in slower and smaller movements to prevent further injury and help healing.

This analysis, however, only considered the presence or absence or the unit and did not consider the possible effect of the pain on the firing rates of those units that were present in the hypertonic saline infusion block as well as the isotonic saline infusion block.

		Slow ramp		Fast Ramp		Step 1		Step 2	
Participant	SMU	VCT	PAM	VCT	PAM	VCT	PAM	VCT	PAM
	1				X	-	-	-	-
	2		X		X	-	-	-	-
	3					-	-	-	-
	4	-	-	X		-	-	-	-
	5	-	-	-	-		X	-	-
	6	-	-	-	-	X			
	7	-	-	-	-	-	-		
5	8	-	-	-	-	-	-		
	9							-	-
	10							-	-
	11	X				-	-		
6	12	-	-	-	-	-	-		
	13							-	-
	14							-	-
	15					-	-	-	-
	16	X		X				-	-
7	17	-	-	-	-	-	-		
	18								
8	19								
	20								
	21						X		
9	22								
	23								
10	24								
	25					X			
	26			X		-	-		
11	27								
	28								
	29								
	30	-	-	-	-	-	-	-	-
	31					-	-	-	-
	32	-	-	-	-			-	-
12	33	-	-	-	-	-	-		
	34	-	-	-	-	-	-		X
	35	-	-	-	-	-	-	-	-
13	36						X		X
	37								
	38		X		X				
	39	-	-	-	-	-	-	-	-
14	40					-	-		
	41	X		X		-	-	-	-

Participant		SLOW RAMP		FAST RAMP		STEP 1		STEP 2	
	SMU	VCT	PAM	VCT	PAM	VCT	PAM	VCT	PAM
	42	X				-	-		
14	43	-	-	-	-	-	-	-	-
16	44					-	-	-	-
	45					-	-		
	46		X		X	-	-		
17	47		X			-	-		
	48		X		X	-	-	-	-
	49		X		X	-	-	-	-
	50	X		X		-	-	-	-
18	51	X		X		-	-	-	-
	52								
	53								
	54					-	-		
19	55		X			-	-	-	-
	56								
	57								
20	58		X			-	-		
Total		6	8	6	6	2	3	0	2

Table 4-60: Shows the 58 units discriminated from the masseter muscle in this study. One "x" was marked when the pattern of recruitment of a certain unit was supportive of one of the models (VCT or PAM), the sign "-" was used when the unit was not present for that specific block, and nothing was marked when the unit was present but their occurrence did not support neither of those models. Note that the majority of the units were neither consistent with the Vicious Cycle Theory nor the Pain Adaptation Model.

According to Table 4-60, it is possible to notice that 6 units supported the Vicious Cycle Theory for the slow ramp jaw closing tasks, 6 units for the fast ramp jaw closing tasks and 2 and 0 units for each of step 1 and step 2 of the 2 step-levels jaw closing tasks respectively. On the other hand, 8 units supported the Pain Adaptation model on the slow ramp jaw closing tasks, 6 units for the fast ramp jaw closing tasks, and lastly 3 and 2 units supported the Pain Adaptation Model for each of step 1 and 2 of the 2 step-levels jaw closing task.

A further analysis was done separately according to the type of task and the number of SMUs recorded per participant, and whether the patterns of recruitments and de-recruitments of all SMUs recorded at a site supported the Vicious Cycle Theory, the Pain Adaptation Model or neither of those models. This analysis is shown in Tables 4-61, 4-62, 4-63, 4-64.

From the 47 SMUs studied in the slow ramp jaw closing task (table 4-61), 70.2% (n=33) of the units did not support neither the Vicious Cycle Theory nor the Pain Adaptation Model, and only 12.8% (n=6) supported the proposals of the VCT and 17% (n=8) supported the proposals of the PAM.

Slow ramp: (Units n = 47)

Participant	Number of Units	VCT	PAM	Neither
5	3		1/3	2/3
6	3	1/3		2/3
7	4	¼		3/4
8	2			2/2
9	3			3/3
10	2			2/2
11	3			3/3
12	3			3/3
13	3			3/3
14	6	2/6	1/6	3/6
16	1			1/1
17	3		2/3	1/3
18	4	2/4	2/4	0/4
19	4		1/4	3/4
20	3		1/3	2/3
Total	47	6	8	33

Table 4-61: Units present for the slow ramp task and consistency or not with the Vicious Cycle Theory (VCT) and the Pain Adaptation Model (PAM). From the 47 units presented for this test, 70.2% (n=33) of the units did not support either the Vicious Cycle Theory nor the Pain Adaptation Model, and only 12.8% (n=6) supported the proposals of the VCT and 17% (n=8) supported the proposals of the PAM.

From the 49 SMUs studied in the fast ramp jaw closing task (table 4-62), 75.6% (n=37) of the units did not support neither the Vicious Cycle Theory nor the Pain Adaptation Model, and only 12.2% (n=6) supported the proposals of the VCT and 12.2% (n=6) supported the proposals of the PAM.

Fast ramp: (Units n= 49)

Participant	Number of Units	VCT	PAM	Neither
5	4	¼	2/4	1/4
6	3			3/3
7	4	¼		3/4
8	2			2/2
9	3			3/36
10	2			2/2
11	3	1/3		2/3
12	4			4/4
13	3			3/3
14	6	1/6	1/6	4/6
16	1			1/1
17	3		1/3	2/3
18	4	2/2	2/2	0/4
19	4			4/4
20	3			3/3
Total	49	6	6	37

Table 4-62: Units presents for the fast ramp task and consistency or not with the Vicious Cycle Theory (VCT) and the Pain Adaptation Model (PAM). From the 49 units presents for this test, 75.6% (n=37) of the units did not support either of the Vicious Cycle Theory nor the Pain Adaptation Model, and only 12.2% (n=6) supported the proposals of the VCT and 12.2% (n=6) supported the proposals of the PAM.

From the 28 SMUs studied in the step 1 of the 2 step-levels jaw closing task (table 4-63), 82.1% (n=23) of the units did not support neither the Vicious Cycle Theory nor the Pain Adaptation Model, and only 7.1% (n=2) supported the proposals of the VCT and 10.8% (n=3) supported the proposals of the PAM.

Step 1: (Units n = 28)

Participant	Number of Units	VCT	PAM	Neither
5	2	½	1/2	0/2
6	2			2/2
7	3			3/3
8	2			2/2
9	3		1/3	2/3
10	2			2/2
11	2	½		1/2
12	3			3/3
13	3		1/3	2/3
14	2			2/2
19	2			2/2
20	2			2/2
Total	28	2	3	23

Table 4-63: Units presents for the step 1 level task and consistency or not with the Vicious Cycle Theory (VCT) and the Pain Adaptation Model (PAM). From the 28 units presents for this test, 82.1% (n=23) of the units did not support either of the Vicious Cycle Theory nor the Pain Adaptation Model, and only 7.1% (n=2) supported the proposals of the VCT and 10.8% (n=3) supported the proposals of the PAM.

From the 35 SMUs studied in the step 2 of the 2 step-levels jaw closing task (table 4-64), 94.3% (n=33) of the units did not support neither the Vicious Cycle Theory nor the Pain Adaptation Model, 0% (n=0) supported the proposals of the VCT and 5.7% (n=2) supported the proposals of the PAM.

Step 2: (Units n= 35)

Participant	Number of Units	VCT	PAM	Neither
5	3			3/3
6	2			2/2
7	1			1/1
8	2			2/2
9	3			3/3
10	2			2/2
11	3			3/3
12	3			3/3
13	3		2/3	1/3
14	4			4/4
17	3			3/3
19	3			3/3
20	3			3/3
Total	35	0	2	33

Table 4-64: Units presents for the step 2 level task. From the 35 units presents for this test, 94.3% (n=33) of the units did not support either of the Vicious Cycle Theory nor the Pain Adaptation Model, and only 0% (n=0) supported the proposals of the VCT and 5.7% (n=2) supported the proposals of the PAM.

4.5 Masseter and temporalis occurrence and consistency with VCT and PAM – a comparison

A further analysis was done to compare the percentage of SMUs with their pattern of occurrence and consistency with the earlier theories mentioned in this study (i.e. VCT or PAM) between the two muscles analysed.

4.5.1 Occurrence

The following tables shows this comparison in the slow ramp and fast ramp jaw closing tasks and step 1 and step 2 of the 2 step-levels jaw closing task for each of the possible effects of hypertonic saline infusion on isotonic saline infusion. That is, where a SMU did not change its occurrence (Table 4-65), where a SMU was recruited during hypertonic saline infusion (Table 4-66), or where a SMU was de-recruited during hypertonic saline infusion (Table 4-67).

	Slow ramp	Fast ramp	Step 1	Step 2
Temporalis	*75.7%*	*74.6%*	*73%*	*82.8%*
Masseter	70.2%	75.6%	82.1%	94.3%

Table 4-65: Summary of SMU data from masseter and temporalis showing percentage of SMUs that did not change their pattern of occurrence between hypertonic and isotonic saline infusion.

	Slow ramp	Fast ramp	Step 1	Step 2
Temporalis	7.2%	12.7%	10.8%	6.9%
Masseter	17%	12.2%	10.8%	5.7%

Table 4-66: Masseter and temporalis as a comparison of recruitment percentage of SMUs that were recruited in the hypertonic saline infusion but not at isotonic saline infusion.

	Slow ramp	Fast ramp	Step 1	Step 2
Temporalis	17.1%	12.7%	16.2%	10.3%
Masseter	12.8%	12.2%	7.1%	5.7%

Table 4-67: Masseter and temporalis as a comparison of recruitment percentage of SMUs that were de-recruited in the hypertonic saline infusion but was present at isotonic saline infusion.

4.5.2 Consistency with VCT and/or PAM

The following table 4-68 shows the comparison in the slow ramp and fast ramp jaw closing tasks and step 1 and step 2 of the 2 step-levels jaw closing task for the percentage of SMUs whose pattern of occurrence were either consistent with earlier theories or not (i.e. did not support either of them, did support the VCT or did support the PAM).

	Slow ramp M	Slow ramp T	Fast ramp M	Fast ramp T	Step 1 M	Step 1 T	Step 2 M	Step 2 T
Did not support either theory	70.2%	80%	75.6%	75.7%	82.1%	73%	94.3%	82.8%
Supported VCT	12.8%	17.1%	12.2%	12.8%	7.1%	16.2%	0%	10.3%
Supported PAM	17%	7.1%	12.2%	12.8%	10.8%	10.8%	5.7%	6.9%

Table 4-68: Summary of SMU data from masseter and temporalis as a comparison of the percentage of SMUs that did not support either theory, supported the Vicious Cycle Theory (VCT) or supported the Pain Adaptation Model (PAM). M= masseter. T= temporalis.

4.6 Associations between psychological variables and jaw muscle activity patterns during pain

4.6.1 Temporalis

A qualitative analysis was done to determine a possible correlation between a change in SMU characteristics (occurrences) between isotonic saline and hypertonic saline infusion, and psychological variables- See tables 4-69 and 4-70. The PCS and DASS-21 scores of the individuals where the occurrence of SMU activity did not alter during any of the infusions for the temporalis muscle (participants 4, 11, 18) were compared with the same scores of those participants where the occurrence of SMU activity did change in at least one block of infusion (participants 1, 2, 5, 6, 7, 10, 12, 13, 14, 15, 16, 17, 19).

	Stress	Depression	Anxiety
DASS-21 for participants that did not change	2.3	0.33	1
DASS-21 for participants who had some change	1.5	0.3	0.4

Table 4-69: DASS-21 scores comparison between participants who had no changes of the SMU occurrence between all the blocks and participants who had.

	Rumination	**Magnification**	**Helplessness**	**Total**
PCS for participants that did not change	8.3	3.3	7.7	19.3
PCS for participants who had some change	2.5	1.7	2.8	6.9

Table 4-70: PCS scores comparison between participants who had no changes on the SMU occurrence between all the blocks and participants who had.

It is possible to notice qualitatively those participants, who did not exhibit a change in the occurrence of their SMUs, had higher scores for all the categories in DASS-21 questionnaire and on the PCS questionnaire. However, the population of participants

that exhibited no change in the SMU occurrence was small (n=3) and the firing rates or threshold values of the units was not considered for this matter.

T-tests were done to compare the scores of the two groups of participants (group 1: participants who had no change on their occurrence of SMU; group 2: participants who had some change on their occurrence of SMU).

No significant differences were found for each score of the DASS-21, Stress (p=0.5), Depression (p=1), Anxiety (p=0.3). However, significant differences were found for 3 scores of the PCS, namely, Rumination (p=0.003), Helplessness (p=0.017), and Total (p=0.009), while only the score of Magnification was not considered significant different (p=0.149).

4.6.2 Masseter

A quantitative analysis was done to determine a possible association between a change in SMU characteristics (in terms of occurrences) and psychological variables – See tables 4-71 and 4-72. The PCS and DASS-21 scores of the individuals where the occurrence of SMU activity did not alter during any of the infusions for the masseter muscle (participants 8, 9, 10, 16) were compared with the same scores of those participants where the occurrence of SMU activity did change in at least one block of infusion (participants 5, 6, 7, 11, 12, 13, 14, 17, 18, 19, 20).

	Stress	Depression	Anxiety
DASS-21 for participants that did not change	0.5	0	0
DASS-21 for participants who had some change	2	0.2	0.5

Table 4-71: DASS-21 scores comparison between participants who had no changes of the SMU occurrence between all the blocks and participants who had.

	Rumination	**Magnification**	**Helplessness**	**Total**
PCS for participants that did not change	3.5	2.25	3.5	9.25
PCS for participants who had some change	2.9	2.1	3.54	8.5

Table 4-72: PCS scores comparison between participants who had no changes on the SMU occurrence between all the blocks and participants who had.

It is possible to notice qualitatively that participants who had not changed the occurrence of their SMUs, had lower scores for all the categories in DASS-21 questionnaire and 3 higher scores (rumination, magnification and total) and 1 higher score (helplessness) on the PCS questionnaire. However, the population of participants that did not exhibit a change in the SMU occurrence was small (n=4) and the firing rates or thresholds values of the units was not considered for this matter.

T-tests were done to compare the scores of the two groups of participants (group 1: participants who had no change on their occurrence of SMU; group 2: participants who had some change on their occurrence of SMU).

No significant differences were found for each score of the DASS-21, Stress (p=0.2), Depression (p=0.4), Anxiety (p=0.3) and for the 4 scores of the PCS, Rumination (p=0.8), Magnification (p=0.9), Helplessness (p=1.0), Total (p=0.9).

4.6.3 Temporalis x masseter

It is possible to notice qualitatively that those participants who had not changed the occurrence of their SMUs, had higher scores for all the categories in DASS-21 questionnaire and on the PCS questionnaire. However, the population of participants that exhibited no change in the SMU occurrence was small (n=3) for the temporalis and for the masseter (n=4) and the firing rates of the units was not considered for this matter. Tables 4-73 and 4-74 summarize a comparison between the masseter and temporalis muscle.

DASS-21:

NOT CHANGE x CHANGE	Masseter	Temporalis
Stress	not significant (p=0.2)	not significant (p=0.5)
Depression	not significant (p=0.4)	not significant (p=1)
Anxiety	not significant (p=0.3)	not significant (p=0.3)

Table 4-73: comparison of participants who had not changed the occurrence of their SMUs with participants who had for each score of the DASS-21 and its significant difference. Not that no significant difference was found for either masseter or temporalis muscle.

PCS:

NOT CHANGE x CHANGE	Masseter	Temporalis
Rumination	not significant (p=0.8)	**Higher and significant** (p=0.003)
Magnification	not significant (p=0.9)	not significant (p=0.149)
Helplessness	not significant (p=1.0)	**Higher and significant** (p=0.017)
Total	not significant (p=0.9)	**Higher and significant** (p=0.009)

Table 4-74: Comparison of participants who had not changed the occurrence of their SMUs with participants who had for each score of the PCS and its significant difference. Not that no significant difference was found for masseter but three scores were significant different for temporalis muscle being higher for participants that did not change their SMU occurrence.

5 DISCUSSION

5.1 Main findings of thesis in relation to the hypotheses

5.1.1 Firt hypothesis

The first hypothesis of our study stated that “experimental noxious stimulation of the right masseter muscle, in comparison with control, does not modify the ability of individuals to execute isometric jaw-closing tasks.”

The findings of the present study are consistent with the first hypothesis. Thus, it was shown that experimental noxious stimulation of the right masseter muscle, in comparison with control, did not modify the capability of individuals to execute isometric jaw-closing tasks. There was no significant effect of the experimental pain on the generation or fine control of the isometric jaw-closing tasks in terms of rates of force increase during fast and slow ramp jaw closing tasks and force amplitudes at the 2 step levels in the 2 step-levels jaw closing task. These findings are in accordance with our first hypothesis.

5.1.2 Second hypothesis

The second hypothesis of our study stated that “experimental noxious stimulation of the right masseter muscle results in significant ($p<0.05$) changes in the activity patterns (i.e. SMU recruitment patterns and thresholds) in the right temporalis muscle (a non-

painful synergistic muscle) and right masseter muscle during isometric ramp jaw closing tasks at two different rates of force increase (slow and fast)."

The findings of the present study are consistent with the second hypothesis. Thus, it was shown that there were indeed some changes in the occurrences of SMU for the temporalis muscle during the hypertonic saline infusion in comparison with the isotonic saline infusion; however, the majority of the units did not change their occurrence in the ramp tasks. In fact, by comparing the hypertonic saline infusion and the isotonic saline infusion for the slow ramp jaw closing tasks, 75.7% of the units did not change their pattern of occurrence, while 17.1% of the units were recruited for the hypertonic saline block of infusion and 7.2% of the units were de-recruited for the hypertonic saline block of infusion. For the fast ramp jaw closing task, 74.6% of the units did not change their pattern of occurrence while 12.7% of the units were recruited for the hypertonic saline block of infusion and 12.7% of the units were de-recruited for the hypertonic saline block of infusion in comparison with the isotonic saline infusion block. No significant changes in the SMU threshold were noted between the hypertonic saline infusion and the isotonic saline infusion blocks during the isometric ramp jaw closing tasks at two different speeds (slow and fast).

In regards to the masseter muscle, similar recruitments patterns were found where the majority of the units did not change their occurrence in the ramp tasks. In fact, by comparing the hypertonic saline infusion and the isotonic saline infusion for the slow ramp jaw closing tasks, 70.2% of the units did not change while 12.8% of the units were recruited for the hypertonic saline block of infusion and 17% of the units were de-recruited for the hypertonic saline block of infusion. For the fast ramp jaw closing task, 75.6% of the units did not change while 12.2% of the units were recruited for the

hypertonic saline block of infusion and 12.2% of the units were de-recruited for the hypertonic saline block of infusion in comparison with the isotonic saline infusion block.

5.1.3 Third hypothesis

The third hypothesis of our study stated that “experimental noxious stimulation of the right masseter muscle results in significant ($p<0.05$) changes in the activity patterns (i.e. SMU recruitment patterns and firing rates of single motor units, and root mean square EMG activity) in the right temporalis muscle (a non-painful synergistic muscle) and right masseter muscle during isometric 2 step-levels jaw closing tasks.”

The findings of the present study are consistent with the third hypothesis. The findings of the present study indeed found some changes in the occurrences of SMUs for the temporalis muscle during the hypertonic saline infusion in comparison with the isotonic saline infusion; however, the majority of the units did not change their occurrence in the 2 step-levels jaw closing tasks. In fact, by comparing the hypertonic saline infusion and the isotonic saline infusion in the 2 steps of the 2 step-levels jaw closing tasks, 73% and 82.8% of the units did not change for step 1 and step 2 respectively. For the step 1, 16.2% of the units were recruited for the hypertonic saline infusion block and 10.8% of the units were de-recruited for the hypertonic saline infusion block. For the step 2, 10.3% of the units were recruited for the hypertonic saline infusion block and 6.9% of the units were de-recruited for the hypertonic saline infusion block. No significant changes in the root mean square of EMG activity or SMU firing rates between the hypertonic saline infusion and the isotonic saline infusion blocks were found at each step level of the isometric 2 step-levels jaw closing tasks.

In regards to the masseter muscle, similar recruitments patterns were found where the majority of the units did not change their occurrence in the 2 step-levels jaw closing tasks. In fact, by comparing the hypertonic saline infusion and the isotonic saline infusion in the 2 steps of the 2 step-levels jaw closing tasks, 82.1% and 94.3% of the units did not change for step 1 and step 2 respectively. For the step 1, 7.1% of the units were recruited for the hypertonic saline infusion block and 10.8% of the units were de-recruited for the hypertonic saline infusion block. For the step 2, 0% of the units were recruited for the hypertonic saline infusion block and 5.7% of the units were de-recruited for the hypertonic saline infusion block. No significant changes in the root mean square of the EMG activity from the masseter muscle were found at each step level of the isometric 2 step-levels jaw closing tasks.

5.1.4 Fourth hypothesis

The fourth hypothesis of our study stated that “experimental noxious stimulation of the right masseter muscle results in significant ($p<0.05$) changes in the activity patterns (i.e. recruitment patterns) in the right masseter (painful muscle) and in the right temporalis muscle (a non-painful synergistic muscle) that are not consistent with earlier theories of pain-motor interaction, namely, the Vicious Cycle Theory and The Pain Adaptation Model.”

The findings of the present study are generally consistent with the 4th hypothesis. Thus, the findings of the present study were mostly not consistent with the earlier models of pain-motor interactions, namely, the Pain Adaptation Model and the Vicious Cycle Theory. There were examples of de-recruitments of SMUs in the temporalis

muscle during hypertonic saline infusion in comparison with isotonic saline infusion (5 SMUs out of 70 SMUs for slow ramp, 9/71 for fast ramp, 4/37 for step 1 of the 2 step-levels and 4/58 for the step 2 of the 2 step-levels jaw closing task) and examples of de-recruitments of SMUs in the masseter muscle (8/47 for slow ramp, 6/49 for fast ramp, 3/28 for step 1 of the 2 step-levels and 2/35 for the step 2 of the 2 step-levels jaw closing task). As these muscles are agonists in these tasks, these observations are consistent with the Pain Adaptation Model that proposes that during pain, agonist muscle activity is reduced in the generation of forces in an attempt to minimize the pain and this inhibition operates at the brainstem level by inhibiting reflex circuits and/or by modifying the central pattern generator for mastication (Lund et al. 1991). There were also examples of recruitments of SMUs in the temporalis muscle during hypertonic saline infusion in comparison with isotonic saline infusion (12/70 for slow ramp, 9/71 for fast ramp, 6/37 for step 1 of the 2 step-levels and 6/58 for the step 2 of the 2 step-levels jaw closing task) and in the masseter muscle (6/47 for slow ramp, 6/49 for fast ramp, 2/28 for step 1 of the 2 step-levels and 0/35 for the step 2 of the 2 step-levels jaw closing task). These observations are indeed consistent with the proposals of the Vicious Cycle Theory which proposes that pain causes a so-called muscle "hyperactivity" and this would be reflected in increases in EMG activity.

5.1.5 Fifth hypothesis

The fifth hypothesis of our study stated that "experimental noxious stimulation of the right masseter muscle results in significant ($p<0.05$) changes in the activity patterns in the right temporalis muscle and right masseter muscle that are associated with the scores from some psychological measures."

The findings of the present study are consistent with the fifth hypothesis. A qualitative analysis of the psychological variables was carried out. The PCS and DASS-21 scores of the individuals where the occurrences of SMU activity did not alter during any of the infusions for the temporalis muscle (participants 4, 11, 18), were compared with the same scores of those participants where the occurrences of SMU activity did change in at least one block of infusion (participants 1, 2, 5, 6, 7, 10, 12, 13, 14, 15, 16, 17, 19). These analyses showed no significant differences for each score of the DASS-21, namely, Stress (p=0.5), Depression (p=1), Anxiety (p=0.3). However, significant differences were found for the PCS, namely, Rumination (p=0.003), Helplessness (p=0.017), and PCS Total score (p=0.009), while only the score of Magnification was not significantly different (p=0.149). A similar comparison for the individuals where the occurrences of SMU activity did not alter during any of the infusions for the masseter muscle showed that there were no significant differences for each score of the DASS-21, namely, Stress (p=0.2), Depression (p=0.4), Anxiety (p=0.3) and for the 4 scores of the PCS, namely Rumination (p=0.8), Magnification (p=0.9), Helplessness (p=1.0), Total (p=0.9).

5.1.6 Summary

Taking all the findings together, the data suggest that experimental masseter muscle noxious stimulation in healthy adults is insufficient to prevent the performance of a set of standardized closing tasks and also does not appear to result in changes in the overall level of EMG activity (in terms of RMS activity) at one site within the masseter muscle and at one site within the temporalis muscle. The data also indicate that, during the noxious stimulation in comparison with control (i.e. baseline or isotonic saline

infusion), most single motor units at a site within the masseter and at a site within the temporalis muscle appear to be recruited during the task under both noxious stimulation and control conditions. In addition, there appears to be no overall effect on SMU thresholds during the ramp tasks, and no effect on SMU firing rates during the step 1 and 2 of the 2 step-levels jaw closing task. However, although the majority of the units were recruited in the tasks during the noxious stimulation block and during the control blocks, there was evidence at both muscle sites, during the noxious stimulation in comparison with control, for recruitment of new SMUs as well as de-recruitment of SMUs during all tasks. While there were no overall significant effects on firing rates, individual SMUs could show small increases or decreases in firing rate during hypertonic saline infusion in comparison with isotonic saline infusion. Further, there was some preliminary evidence, at least for the temporalis muscle, that the changes in the recruitment patterns of SMU activity occurred in those individuals who had PCS scores significantly lower than those individuals who did not show any evidence of changes in recruitment patterns. These data provide some support for the observation that psychological factors may be playing a role in the pain-motor interaction.

The effects noted in terms of recruitments/de-recruitments were not supportive of earlier models of pain-motor interaction (namely, Vicious Cycle Theory, Pain Adaptation Model). Rather the data point towards more complex effects of pain on motor activity which suggest that a reorganization of motor unit recruitments occurs both within the painful muscle, as has also been demonstrated for the masseter muscle in this present thesis and confirming previous work (Malik 2016; Minami et al. 2013), and in addition, as demonstrated in this present work, within other non-painful muscles.

The reorganization appears to involve a recruitment of one population of SMUs and a de-recruitment of another population of SMUs.

There is good evidence that all the jaw closing muscles (namely, bilateral masseter, temporalis and medial pterygoid) are involved in the generation and control of the tasks employed in the present thesis (Hannam and McMillan 1994; Miller et al. 1982). The present data supporting a reorganization of SMU activity within non-painful muscles suggest that re-organization is not restricted to the painful jaw muscle but that the entire jaw motor system may undergo a re-organization of SMU recruitment patterns, and possibly firing rates, to allow successful task performance. Further, there is suggestive evidence that this reorganization might be influenced by the level of pain catastrophizing with those with higher PCS scores possibly exhibiting less ability to undergo re-organization of SMU activity, that is, these individuals with higher PCS scores did not exhibit a change in SMU recruitment patterns between hypertonic saline and isotonic saline infusion. The findings also provide another explanation as to how task dynamic features (i.e. force rates and force levels) can be unaffected in pain, that is, a reorganization of SMU activity may be operating in other agonists to the task and not just the agonist subjected to the noxious stimulus.

These new data point to newer models as explanations for the effects of pain on motor activity. One such model is the Integrated Pain Adaptation Model, which considers the interaction of the individual's biopsychosocial characteristics with the individual's pain experience (i.e., the multidimensional nature of pain) and the anatomical and functional complexity of the individual's sensory-motor system.

5.2 Demographics, questionnaires and infusion data:

5.2.1 RDC/TMD

The first effort at an evidence-based diagnostic method for TMDs was the Research Diagnostic Criteria for TMD (RDC/TMD) in 1992 (Dworkin and LeResche 1992) and is nowadays still a diagnostic system widely used in the literature (Fernandes Azevedo et al. 2017; Goiato et al. 2017; List and Dworkin 1996; Minami et al. 2013; Osiewicz et al. 2017; Sae-Lee et al. 2006; Yap et al. 2002). The RDC/TMD came from the accepted need for a diagnostic system that could distinguish cases from controls for epidemiological and clinical research purposes, and also differentially define and diagnose common subtypes of chronic pain–related TMDs (Ohrbach and Dworkin 2016). Although the new DC/TMD (Schiffman et al. 2014) is available, the RDC/TMD was used in this present study, and all the participants were classified as free of TMD.

5.2.2 DASS21

The DASS 21 is a reliable questionnaire, besides the fact that is free to use and brief to administer (Nilges and Essau 2015). Its reliability is widely demonstrated in the literature (Le et al. 2017; Osman et al. 2012; Tonsing 2014), and its validation in several languages is readily found on the literature as, for example, Chinese (Wang et al. 2016), Arabic (Ali et al. 2017), Brazilian and Portuguese (Vignola and Tucci 2014).

Assessing the severity of the core symptoms of Depression, Anxiety and Stress showed that all of the participants in the current study had low scores for each of the scales. The highest mean values were for stress (1.5, SD: 1.9), followed by anxiety (0.5, SD: 0.7) and then depression (0.25, SD: 0.8).

Recent studies (Ajilchi and Nejati 2017; Lei et al. 2015) have used a cut-off point where a patient would be considered positive for depression if their DASS-21 depression item score was ≥14, anxiety if their DASS-21 anxiety item score was ≥10, and stress if their DASS-21 stress item score was ≥19. All the scores in this study were lower than that.

Besides, the range of scores in this study (from 0.25 to 1.5) were lower than TMD chronic patient scores according to a previous report from a large sample of chronic pain patients and which exhibited a range of scores from 9 to 14 (Nicholas et al. 2008). However, one limitation is the fact that this study evoked noxious stimulation in healthy individuals and the comparison with chronic pain patients is limited. Yet, all participants in this study were not clinically depressed, anxious or distressed.

5.2.3 PCS

Considerable evidence has linked pain catastrophizing to pain responses, and recent experimental pain research has suggested that situational catastrophizing, measured during or immediately after laboratory pain procedures, is strongly related to pain ratings of standardized noxious stimuli (Campbell et al. 2010).

In fact, one study published in 2016, manipulated healthy participants and chronic tension-type headache patients by giving them 3 types of hypnotic suggestions: Negative (based on the 13 items in the Pain Catastrophizing Scale), Positive (coping-oriented reversion of the Pain Catastrophizing Scale), and Neutral (neutral sentences). This study reported that a change in pain catastrophizing predicted changes in pain in patients and in healthy volunteers (Kjogx et al. 2016).

In another study (Akhter et al. 2014), experimental muscle pain was induced by hypertonic saline infusion into the right masseter muscle. This study showed that, in comparison with lower pain catastrophizers, individuals with higher pain catastrophizing scores exhibited significant greater values for pain intensity and unpleasantness intensity ratings, for all the scores of the MPQ, for the perceived area of pain and for the number of referral sites for pain.

Pain catastrophizing is widely shown to be associated with poor pain treatment responses in patients with chronic pain (Edwards et al. 2006; Haythornthwaite et al. 2003; Mankovsky et al. 2012). However, pain catastrophizing is malleable and responsive to manipulation (Darnall et al. 2017) and levels of catastrophic thinking have been shown to vary in relation to exposure to a wide range of experimental or clinical pain stimuli (Campbell et al. 2010).

However, research at the University Centre for Research on Pain and Disability indicates that a total PCS score of 30 represents a clinically relevant level of catastrophizing (Sullivan MJL 1995) and the total mean score in this study was low at 8.35 and within the 3 subscales, rumination and helplessness had the highest group mean (3.25) for both followed by magnification (1.85). Therefore, our data support the statement that all participants had low pain catastrophizing levels. It is important to mention that the variability in the PCS scores between participants was found which is in accordance with previous studies (Campbell et al. 2010; Hsieh et al. 2010; Kristiansen et al. 2014) and this can at least partially explain the high variability in the pain ratings scores with different infusion rates found in this study. This is in accordance with a previous study that stated that even small increments in pain catastrophizing score can influence pain perception to deep and tonic stimulations (Kristiansen et al. 2014).

5.2.4 Infusions

5.2.4.1 Use of the hypertonic saline

In the present study, 5% hypertonic saline solution was successfully infused with the aid of an infusion pump into the central region of the right masseter muscle in 20 participants.

The induction of a noxious stimulation of a muscle with the use of hypertonic saline infusion is widely used in a large number of research studies in the orofacial area (Akhter et al. 2014; Christidis et al. 2008; Dagsdottir et al. 2015; Inamoto et al. 2017; Minami et al. 2013; Shimada et al. 2013; Wiesinger et al. 2013), limb muscles (Castelein et al. 2017; Rice et al. 2015; Salomoni et al. 2016), neck muscles (Christensen et al. 2017; Gizzi et al. 2015; Lindelof et al. 2009) and lower back (Danneels et al. 2016; Larsen et al. 2017; Smith et al. 2005) as it produces a local area of transient pain similar in quality and intensity to clinical myalgia (Capra and Ro 2004).

Although other substances might be used to induce pain in experimental studies, such as monosodium glutamate (Costa et al. 2017; Kumar et al. 2015a; Pasinato et al. 2016), or capsaicin (Arima et al. 2001; Romaniello et al. 2000), a major reason for the common use of hypertonic saline is that virtually no side effects are reported after hypertonic saline injection, so it can be considered safe (Christidis et al. 2008). And, in fact, none of the participants in the present study reported any undesirable side effects during and after the infusion.

Another reason for its widespread use of the hypertonic saline in laboratory pain studies is that the quality of the induced pain by hypertonic saline is comparable to acute clinical muscle pain and shows localized and referred pain characteristics (Feinstein et al. 1954; Svensson et al. 1995).

It has been reported that injection of hypertonic saline into a jaw muscle results in an increase of pain to reach its maximum intensity, and then it decreases shortly after the infusion is stopped and declines to no pain within 5-10 minutes (Svensson and Arendt-Nielsen 1995). These temporal features were observed in the present study. The mechanisms involved in the initiation of the saline-induced pain, however, are not completely understood. A direct activation of nociceptors and the release of neuropeptides and other mediators have been suggested (Tegeder et al. 2002).

In this earlier study (Tegeder et al. 2002), the release of algesic substances in human experimental muscle pain after 2 sets of 50 concentric/eccentric contractions was compared after the injection hypertonic saline infusion. They provided evidence that the injection of hypertonic saline into a muscle directly stimulates muscle nociceptors and causes glutamate release, which is strongly associated with muscle pain (Dawson et al. 2013; Louca et al. 2014; Shimada et al. 2016).

Lastly, one study found that the saline infusion probably causes nociceptive and non-nociceptive excitation of muscle receptors. However, the excitation of non-nociceptive muscle afferents is assumed to have a smaller influence on the sensory-motor interactions compared to the excitation of nociceptive muscle afferents (Madeleine et al. 1999).

5.2.4.2 Isotonic infusion as a control

Even though it is unlikely that the volume of infusion would cause any changes in pain perception, as the extracellular fluid formed by the infusion is still present for a few hours after the pain is vanished (Graven-Nielsen 2006), this study used 0.9% isotonic saline infusion as a control for possible changes in the muscle activity due to the volume of infusion. Isotonic saline infusion is a good control for volume as an earlier study found that bolus injections of isotonic and hypertonic saline result in the same increase in intra-muscular pressure (Graven-Nielsen et al. 1997c).

The higher concentration of the hypertonic saline infusion (5%) compared with the lower concentration of the isotonic saline infusion (0.9%) is therefore most likely the reason for the pain generated by the hypertonic saline and that the use of the isotonic saline infusion caused minimal or no pain in the participants of this study. Therefore, the comparison between hypertonic saline infusion and isotonic saline infusion provides an assessment of the net effect of pain on motor activity. This conclusion is also in accordance with previous studies (Amhamed et al. 2016; Castrillon et al. 2017; Minami et al. 2013; Sae-Lee et al. 2006; Sae-Lee et al. 2008a; Svensson et al. 2008; Svensson et al. 1998; Svensson et al. 1997).

Pain has been induced in the human temporalis muscle by the local injection of (1) hypertonic saline and (2) potassium chloride, using isotonic saline as control (Jensen and Norup 1992). This study found that both hypertonic saline and potassium chloride induced significantly more pain than the isotonic saline.

5.2.4.3 *Maintaining perceived pain*

In the present study, the same perceived pain intensity was achieved by using different amounts of saline into the right masseter of different participants. The need for a change in infusion rate of saline was determined after each trial by viewing the VAS score of each participant. If the participant marked a score on a position of the VAS that was visually equivalent to a value lower than 30/100 mm, the infusion rate of hypertonic saline was increased, and consequently more volume of hypertonic saline was infused. Similarly, after a VAS score was marked on a position equivalent to a value higher than 60/100 mm, the infusion rate was decreased.

In the present study, the same range of pain perception (i.e. 30-60/100 mm on the VAS) was maintained by varying the injection rate so that a total volume of from 0.1 to 1.9 ml of 5% hypertonic saline was infused. In fact, inter-individual variations in scores of pain intensity have been reported (Akhter et al. 2014; Amhamed et al. 2016; Graven-Nielsen et al. 1997a; Jensen and Norup 1992; Kumar et al. 2015a; Sae-Lee et al. 2008a) and there is even evidence for intra-individual variability in scores of pain intensity, quality, distribution and sensory cutaneous changes after saline-induced muscle pain (Graven-Nielsen et al. 1997a).

This inter-individual variation might be explained by the influence of an individual's genetic composition, prior learning, current physiological status, idiosyncratic appraisals, expectations, current mood states, and sociocultural environment (Turk 2002). These influences manifest as variability in pain sensitivity, perception and

tolerance (Kim et al. 2004). Gender is also commonly cited as a possible cause for differences in pain perception between males and females (Cairns et al. 2001; Naliboff et al. 2003; Walsh et al. 2017).

5.2.5 VAS

Most of the pain assessment tools used by patients and research participants to self-report their pain are only one dimensional, indicating only pain intensity. They are quick, easy to use, and economical. Examples include the numeric rating scale (NRS), the visual analog scale (VAS), Faces Pain Scale–Revised (FPS-R), Iowa Pain Thermometer (IPT), and Verbal Descriptor Scale (VDS) (Topham and Drew 2017).

The verbal rating scale (VRS), although extensively used, has several disadvantages as compared to the VAS. Indeed, comparing those two assessments, it has been shown (Ohnhaus and Adler 1975) that the VAS seems to assess more meticulously what a patient actually experiences regarding possible changes in the perception of pain intensity. It also appeared to be more satisfactory than the 4-point scale (FPS) for patient self-rating of pain intensity (Joyce et al. 1975).

The visual analogue scale was used in this study to measure pain perception due to its simplicity and rapidity. However, it is important to mention its limitations. For example, a previous study (Williams et al. 2000) has examined the use of simple pain ratings' scales and has shown that such measures are based on narrow considerations and there are possible sources of error. They found that patients with chronic pain had

difficulty in rating pain by a single score and the score would possibly be influenced by, and with reference to, a range of internal and external factors and private meanings (Williams et al. 2000).

In the present study, the mean pain intensity induced by infusion of 5% hypertonic saline was measured with the aid of the VAS and was associated with moderate pain intensity (30-60 mm on 100 mm VAS). The scores obtained are entirely consistent with previous reports of algesic chemical injections into the jaw muscles (Akhter et al. 2014; Amhamed et al. 2016; Svensson et al. 1996b).

Three participants exhibited VAS scores >10/100 mm in one or more tasks of the isotonic saline infusion, but the hypertonic saline infusion scores were higher and therefore, it was considered that the net effect of pain was still being studied in these participants. Most of the isotonic saline infusion scores were at or near 0/100 mm and this is consistent with previous studies that have used isotonic saline injections into the jaw muscles (Graven-Nielsen et al. 1997a; Sae-Lee et al. 2006; Sae-Lee et al. 2008a). The higher pain scores in some participants can possibly be explained by the discomfort of the needle or by the increase of the intramuscular pressure within a confined anatomical compartment of the masseter during the performance of the jaw tasks (Graven-Nielsen et al. 1997b).

5.2.6 Distribution of perceived pain

In the present study, all participants (n=20) described localised pain in the area of the right masseter that was infused with the hypertonic saline solution. However, 2 participants (participant 2 and 17) also reported referred pain in the right anterior temporalis.

Furthermore, for the isotonic saline infusion block, 4 participants (participant 2, 4, 6 and 19) reported localised pain in the area of the right masseter, and one participant (participant 2) also reported referred pain in the right anterior temporalis. However, it was considered that the pain was probably due to discomfort associated with the needle insertion and this is in accordance with a previous study (Semciw et al. 2013) who reported a low level of discomfort after the insertion of intramuscular electrodes into the gluteus medius and gluteus minimus muscle. However, this discomfort was not considered to alter motor behaviour during locomotion in healthy and low back pain patients (Smith and Kulig 2015).

Referred pain can be defined as pain occurring outside and remote from the local pain area (Graven-Nielsen 2006) and is commonly associated with hypertonic saline infusion experiments in different muscles of the human body (Drew et al. 2017; Izumi et al. 2014; Macefield et al. 2007; Rubin et al. 2010; Rubin et al. 2012). In fact, our results are consistent with previous studies (Graven-Nielsen et al. 1997b; Malik 2016; Minami et al. 2013; Sae-Lee et al. 2008b; Stohler et al. 1992; Svensson et al. 1996a) where the infusion of hypertonic saline into the masseter muscle induced pain that was localized at the injection site, but also the pain could be referred to the adjacent areas

of the temporal region, in or around the TMJ, ear, or sometimes referred to the posterior teeth or mandible.

In their study, Jensen and Norup induced pain in 20 healthy subjects with 0.2 ml pain-inducing solution injected into one temporal muscle and isotonic saline into the other. Forty-eight percent of the injections led to the referral of pain most often to the jaws. A positive correlation between the relative occurrence of referred pain and pain intensity was observed ($p < 0.001$) (Jensen and Norup 1992). Injection of hypertonic saline in the tibialis anterior (TA) produced referred pain in the ankle (Graven-Nielsen et al. 1997b). Hypertonic saline injection into an intrinsic neck muscle induced trunk and axioscapular muscle referred pain (Christensen et al. 2015).

Referred pain after noxious muscle stimulation is more likely to depend primarily on central mechanisms (Sessle 2006). This phenomenon of referral pain is a common clinical find 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 neurones (Feinstein et al. 1954; Sessle 2006). Extensive convergent input from TMJ, muscle, and tooth pulp afferents via craniofacial nerves to cutaneous nociceptive neurones onto second order neurones in subnucleus caudalis could help explain the poor localization and referral and spread of pain involving the deep musculature (Sessle 2006). Furthermore, the development of new receptive fields due to central sensitization could mediate referred pain (Mense et al. 2001).

5.2.7 McGill Pain questionnaire

Multidimensional scales that include sensory, affective, and evaluative aspects are used in outpatient settings for the management of persistent pain. Examples include the McGill Pain Questionnaire, Chronic Pain Grade Scale, Short Form 36 Bodily Pain subscale, and Brief Pain Inventory (Topham and Drew 2017).

In this study, the MPQ was used and the data indicate that hypertonic saline infusion had a greater effect on the evaluative descriptors from the MPQ as compared to the other dimensions. The difference in the MPQ Total pain rating indices indicates a difference between hypertonic (7.37) and isotonic saline (2.26) infusions for the mean scale scores and also a difference between hypertonic (7.89) and isotonic saline (2.54) for the mean weight score.

These results are in agreement with a number of previous studies that shows significant pain rating index (PRI) differences between hypertonic saline and isotonic saline infusion (Akhter et al. 2014; Malik 2016; Sae-Lee et al. 2006).

Although some studies found some differences in the MPQ values for acute pain compared with real chronic pain patients (Reading 1982; Wilkie et al. 1990), 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 and Kowalski 1999).

The most cited word in the present study for the hypertonic block of infusion was “annoying” (14/20) followed by “aching” (13/20), “pressing” (12/20) and “jumping” (8/20). “Boring” was cited by 3 people and “annoying” by 2 during the isotonic block of infusion. “Annoying” was also the most cited word in a study involving hypertonic saline infusion into trunk muscles (Hirata et al. 2015). “Aching” was also one of the most cited words from previous studies in the jaw motor system (Malik 2016; Sae-Lee et al. 2008a). “Pressing” was also widely cited in another study (Kumar et al. 2015a).

However, it is important to mention that the quality of pain and its impact using verbal descriptors are dependent on the individual's verbal repertoire. Incomprehension of the descriptors (Main 2016) and ambiguity of usage (Fernandez and Towery 1996) may be a factor influencing the words chosen.

5.2.8 Advantages and limitations of Infusion models

The relationship between clinical phenomena and the diverse findings of studies performed in animal and human models of experimental pain is often obscure (Portenoy 1989), and it can be difficult to translate the findings from experimental studies to the clinical situation.

One ideal experimental pain stimulus should not be invasive, and should not produce tissue damage; should be specific for measure pain and no other sensations; should

be measurable, and demonstrate a relation between the noxious stimulus and the pain intensity; should vary from zero to maximal tolerable levels and lastly should be reproducible and easily repeatable with no change in the response over time (Arendt-Nielsen and Sumikura 2002; McCain 1987).

However, according to Arendt-Nielsen and Sumikura in 2002, we cannot directly measure pain. We can, instead, measure different components which together are important for the pain experienced. In the same study they also compared animal and human experimental pain studies and they state that the perceived pain intensity and quality that can be recorded in awake humans is the main advantage when compared to animal models. In animals, on the other hand, invasive techniques that are not possible to reproduce in humans, such as direct recordings from the spinal cord dorsal horn, can be applied (Arendt-Nielsen and Sumikura 2002).

Experimental pain models have been used to demonstrate local and referred pain but the more significant clinical dilemma is diagnosis and treatment of chronic muscle pain. Important elements in the establishment of chronic pain are peripheral and central sensitization with signs of muscle hyperalgesia and allodynia (Capra and Ro 2004). However, single hypertonic injections in leg muscles did not produce significant muscular or subcutaneous hyperalgesia in experimental pain studies (Graven-Nielsen et al. 1997d) and therefore make it difficult to translate to clinical pain states.

Nonetheless, experimental pain models have contributed to our understanding of central hypersensitivity in different chronic pain conditions. For example, Koelbaek

Johansen and co-workers, for instance, demonstrated that not only the pain was induced by hypertonic intramuscular saline but also the area of referred pain was significantly increased in whiplash patients compared to controls, which can possibly be explained by a central sensitization in those patients (Koelbaek Johansen et al. 1999).

While the findings of the present thesis only directly apply to a group of young healthy individuals experiencing a brief experimental pain stimulus, a cautious extrapolation of the findings may be made possibly to individuals experiencing an acute episode of pain.

5.3 Effects of pain on force rate and amplitude

In the present study, there was no significant effect of the blocks (baseline, hypertonic saline infusion or isotonic saline infusion block) on the force amplitudes (N) in the slow ramp and the fast ramp jaw closing tasks or the force levels (N) achieved at each step level (step 1 and step 2) of the 2 step-levels jaw closing task. Therefore, despite the presence of mostly moderate levels of pain intensity, all individuals were able to perform all the tasks in this study and were able to maintain and reproduce the same force levels across all the blocks analysed. These findings are not consistent with the earlier proposals of the Pain Adaptation Model which would indicate a pain-induced reduction in force rates and/or force levels during the performance of the tasks.

The isometric jaw closing task in the present study is likely driven by the primary motor cortex (Avivi-Arber et al. 2011; Avivi-Arber and Sessle 2017; Sessle 2006), and there is good evidence in the spinal and trigeminal literature that the primary motor cortex is inhibited by noxious stimulation (Adachi et al. 2008; Nash et al. 2010; Svensson et al. 1996a). It has been observed, for example, that noxious stimulation of the tongue inhibited the excitability of the tongue region of the primary motor cortex but did not affect the excitability of the region driving the digastric muscle (Adachi et al. 2008). It is possible that localised noxious stimulation within a muscle may selectively inhibit the region of the primary motor cortex driving the motor units in the region of the noxious stimulation. While this is speculative, the present findings of an absence of an effect of noxious masseter stimulation on biting task performance, possibly indicates that irrespective of pain in the masseter muscle, the primary motor cortex may undergo rapid neuroplastic changes to allow for the task to be completed with equal force production during the hypertonic saline infusion as during the isotonic saline infusion. This is also part of the neuroplasticity in the system with the possibility of rapid changes being able to occur in the recruitment patterns of motor units in association with the noxious stimulation. There are many motor units that are available in the jaw closing muscles that can be quickly recruited to contribute forces to allow the tasks performed in the present study in the presence of pain.

Cortical neuroplastic changes have been associated with altered motor function or behaviour in other orofacial motor tasks, such as that which occurs following the acquisition of novel motor-skills Boudreau et al., 2007; Boudreau et al., 2010). Some of these cortical and/or behavioural effects can be modified by orofacial pain (Boudreau et al. 2007, 2010; Kumar et al. 2015). These neuroplastic changes may

result in increases or decreases in SMU activity at the site and at other sites and muscles involved in task performance. These higher centre influences may contribute to the modifications of EMG activity that have been observed in experimental and clinical pain (Lund and Stohler 2007; Mense 2007; Murray and Peck 2007; Svensson 2007; van Dieen et al. 2003). These suprabulbar influences have been incorporated into a more recent model of pain-motor interaction, the Integrated Pain Adaptation Model (Murray and Peck 2007). This model suggests that during a painful condition, there is a reorganization of activity within muscles, which may involve not only any of the muscles that might be in pain but also the non-painful muscles. These changes in activity occur so as to maintain motor function and the performance of necessary kinematic-tasks (Svensson et al. 1996a). The EMG findings of the present study support this conclusion.

Another possible reason for the lack of effect of pain on task performance could relate to the fact that the pain, induced by the hypertonic saline solution, is known by the participant to be short lasting even though it has intensity entirely comparable to that noted in patients with clinical TMD pain (Castrillon et al. 2008; Gustin et al. 2011). All the individuals tested were also healthy young individuals. Therefore, the knowledge and selection of the participants is likely to have been a factor in influencing the effects observed, for example, motivational aspects may have meant that the individuals were able to perform the task as well under hypertonic saline infusion as isotonic saline infusion. However, individuals who have a range of risk factors for TMD (Maixner et al. 2011; Slade et al. 2007), may exhibit a different motor effect with the experience of a brief episode of noxious stimulation; such individuals may have demonstrated significantly reduced bite force parameters. This is a possible avenue for further study.

Another possible reason for the absence of a pain effect in the force generation in this present study could relate to the fact that the tasks were not performed at maximal force generation. The lower forces employed may not be able to demonstrate the effect that pain has on the force generation as proposed by the Pain Adaptation Model.

Note that while there was no overall effect of the pain on these dynamic measures of the tasks, there was suggestive evidence of individual variability (see Tables 4-17, 4-18, 4-21, 4-22) with some individuals showing increases in dynamic variables in pain (vs control) while others showed decreases. It remains to be determined whether these variations reflect individual pain-related effects. Individual variations in jaw kinematic or dynamic features during hypertonic saline infusion in comparison with isotonic saline infusion have been previously reported in isometric jaw closing tasks (Malik 2016) and in standardized jaw movements and in free and standardized chewing (Amhamed et al. 2016; Sae-Lee et al. 2008a).

In fact there are many studies providing data that are in accordance with the view that experimental orofacial pain does not change the ability of individuals to perform a jaw motor task (Amhamed et al. 2016; Michelotti et al. 2014; Minami et al. 2013; Sae-Lee et al. 2008a; Salomoni and Graven-Nielsen 2012). Limb or trunk motor studies in pain also have reported that a motor task can be performed via different patterns of muscle activation, but without a change in the ability to perform a motor task (Christensen et al. 2017; Falla et al. 2008; Gizzi et al. 2015; Hodges and Moseley 2003).

There are also a number of studies in the jaw motor system that demonstrate only minimal or no effects on jaw kinematics or dynamics of experimental pain (Amhamed et al. 2016; Gizzi et al. 2015; Kumar et al. 2015a; Malik 2016; Michelotti et al. 2014; Minami et al. 2013; Sae-Lee et al. 2008a; Sohn et al. 2004; Svensson et al. 1996a; Svensson and Graven-Nielsen 2001; Wang et al. 2000) but also of clinical pain (Brandini et al. 2011; Pereira-Cenci et al. 2007; Stohler et al. 1988) – see (Murray and Peck 2007) for review. For example, to test the hypothesis that experimental pain in the masseter muscle or temporomandibular joint would decrease the anterior maximum voluntary bite force (MVBF), experimental pain was shown not to affect the MVBF, which was also in accordance with the subject-based reports (Kumar et al. 2015b). A clinical study comparing TMD patients with healthy control individuals also demonstrated no differences in maximal bite force between the TMD and the control group (Pereira-Cenci et al. 2007).

Not all studies report no or only minimal effects of pain on jaw kinematic or dynamic variables. For example, a recent study evaluating the changes in pain and force in patients with muscle pain and bruxism, prior to and after 30 days of treatment (with occlusal splints, patient education, and physiotherapy) provided evidence that the pain level decreased after treatment and this was correlated with a bite force increase in the molar region (Goiato et al. 2017). The findings of the present study for an isometric jaw closing task also contrast with our previous findings of significant effects of experimental masseter muscle pain on the kinematic variables of some jaw movement tasks, such as the jaw opening amplitude during standardized open-close jaw tasks

(Sae-Lee et al. 2008b) and during repetitive open-close jaw movements (Akhter et al. 2014).

Our study is also not consistent with the findings of a previous study where breaking of hard food in a group of masticatory muscle pain patients took a longer time than in a pain-free healthy control group (Shiau et al. 2003). In addition, a study of patients with signs and symptoms of TMD, compared with control, showed higher masticatory efficiency, increased chewing time and increased EMG activity of the masseter and temporalis muscles and featured an altered chewing pattern (Rodrigues et al. 2015). Another study actually found that the TMD patients chewed faster and with higher amplitude jaw movements in comparison with matched controls (Brandini et al. 2011).

The inconsistencies between studies may relate to methodological differences between studies (e.g. dependent variables chosen), or may relate to true differences between tasks as to the effects that pain has on motor activity. This conclusion is consistent with the findings from a review of an extensive analysis of the literature on trunk muscle recruitment in low back pain patients. This extensive review concluded that neither one of the two models examined in this review (namely the Vicious Cycle Theory and The Pain Adaptation Model) adequately predicted the effects of back pain on trunk muscle activation (van Dieen et al. 2003). They also stated that the changes observed are likely to be task-dependent, related to the individual problem and hence highly variable between and probably within individuals. Therefore, more complex models of pain-motor interactions have been previously proposed (Murray and Peck 2007).

5.4 Root Mean Square (RMS) analysis of EMG activity

Root mean square analysis of EMG activity from the masseter and temporalis muscles during the 2 step-levels jaw closing task revealed no significant differences in the level of EMG activity between baseline, hypertonic saline infusion, isotonic saline infusion and baseline 2 from both muscles. Previous studies of experimentally induced pain in humans have shown increases (Del Santo et al. 2007; Sae-Lee et al. 2008a; Sessle 1999b; Svensson et al. 1997), decreases (Del Santo et al. 2007; Farina et al. 2005; Sae-Lee et al. 2008a) or no effects (Farina et al. 2004; Matre et al. 1999; Sae-Lee et al. 2008a; Schulte et al. 2004) on muscle activity.

These RMS findings of the present study are generally inconsistent with many previous studies showing that pain does indeed have effects on overall EMG activity during a variety of tasks (Baad-Hansen et al. 2009; Farina et al. 2005; Graven-Nielsen et al. 1997c; Sae-Lee et al. 2008b; Shimada et al. 2013; Sonnesen and Svensson 2013; Tucker et al. 2009). But the present study is in accordance with a previous study (Malik 2016) that was performed and which used a similar methodology as in the present study and which found no significant effect of pain on RMS activity. Also, no differences have been reported in the RMS EMG activity of muscles contralateral to the muscle where pain was induced and in the same experimental paradigm as performed here (Sandoval 2017). The different experimental paradigms (e.g. the different tasks) for some of these other studies may be a factor contributing to the differing observations of the effects of pain on global EMG activity. Also an earlier study of the effects of

hypertonic saline induced pain on EMG activity during standardized jaw displacements (Sae-Lee et al. 2008a), provided evidence that under constrained goal-directed tasks, the pattern of pain-induced changes in jaw muscle EMG activity is not clear cut, but can vary with the task performed, jaw displacement magnitude, and the subject being studied. For example, this earlier study showed that the effect of experimentally induced muscle pain by hypertonic saline on agonist muscle activity could vary between tasks in comparison with isotonic saline infusion. The activity of the inferior head of the lateral pterygoid muscle was not affected during a contralateral and a protrusive jaw movement but was significantly affected during jaw opening (Sae-Lee et al. 2008).

Although these collective results of RMS EMG activity in the present study did not reveal any significant differences between hypertonic saline and isotonic saline infusions, interestingly, the means were higher in the baseline 2 for the right masseter muscle step 1 and step 2 than all the other blocks within the same muscle, and higher for the hypertonic block for the right temporalis muscle step 1 and step 2 for all the other blocks within the same muscle

These differences might reflect a number of factors. One possible factor is that the pain may have different effects on different jaw muscles and also may be task dependent in that some motor tasks may be more likely to show an effect than other tasks. This conclusion is entirely consistent with previous observations that pain has different effects on the EMG activity of different jaw muscles (Sae-Lee et al. 2008b; Svensson et al. 1997). In fact, Hannam and McMillan in 1994 pointed out other factors that may

explain these differences on the RMS activity such as the location of the SMU, its background firing rate, the timing of the stimulus, and the task used. The last one is a common feature of human jaw SMU behaviour and it reflects interaction between peripheral sensory information from orofacial and muscle afferents and corticobulbar drive (Hannam and McMillan 1994).

Motivation and other higher centre influences might be other factors that could possibly influence the differences in RMS activity in this study. According to the methodology of this study, all the participants were verbally encouraged to follow the target as much as possible. This might have implications as different effects are likely to be observed during pain when participants are instructed to follow a target in comparison to free movements. For the former, the motivation to track the target may override any possible pain-related EMG or movement effect and this has been noted previously (Sae-Lee et al. 2008b).

An individual analysis of RMS EMG activity indicated that potential individual effects of pain on RMS activity might be apparent and where pain could possibly induce different motor effects between individuals. It was possible to classify that changes were either consistent with The Vicious Cycle Theory (VCT) or The Pain Adaptation Model (PAM) within an individual. For example, at the right masseter and at the right temporalis during step 1 and step 2 of the 2 step-levels jaw closing task, there were many examples of an increase in RMS activity which was consistent with VCT as well as many examples of a decrease in RMS activity which was consistent with the PAM during hypertonic saline infusion in comparison with isotonic saline infusion.

These possible effects may simply reflect variation in EMG activity related to, for example, small variations in the force levels achieved in the step tasks. Alternatively, the present analysis suggested that these individual differences in EMG activity between hypertonic saline infusion and isotonic saline infusion actually reflect an individual pain-related effect and was not simply due to the fact that the participant exerted more or less force in a particular block. For the masseter step 1, 10 participants increased their RMS activity during hypertonic saline infusion and among them, 4 presented lower force during the hypertonic saline block and 6 presented lower force during the isotonic saline block. On the other remaining 10 participants, that decreased their RMS activity during the hypertonic saline infusion, 5 presented lower force during the hypertonic saline infusion and 5 presented lower force during the isotonic saline infusion. For step 2, 6 participants increased their RMS activity during hypertonic saline infusion and among them, 5 presented lower force during the isotonic saline block and only 1 presented lower force during the hypertonic saline block. On the remaining 14 participants, that decreased their RMS activity during the hypertonic saline infusion, 6 presented lower force during the hypertonic saline infusion and 8 presented lower force during the isotonic saline infusion.

For the temporalis step 1, 10 participants increased their RMS activity during hypertonic saline infusion and among them, 5 presented lower force during the hypertonic saline block and 5 presented lower force during the isotonic saline block. On the other remaining 10 participants, that decreased their RMS activity during the hypertonic saline infusion, 4 presented lower force during the hypertonic saline infusion and 6 presented lower force during the isotonic saline infusion. For step 2, 9

participants increased their RMS activity during hypertonic saline infusion and among them, 5 presented lower force during the isotonic saline block and 4 presented lower force during the hypertonic saline block. On the remaining 11 participants, that decreased their RMS activity during the hypertonic saline infusion, 3 presented lower force during the hypertonic saline infusion and 8 presented lower force during the isotonic saline infusion.

This suggestive evidence of individual effects in the present study is consistent with previous findings and conclusions (Amhamed et al. 2016; Hodges and Tucker 2011; Sae-Lee et al. 2008a; Sae-Lee et al. 2008b; Wiesinger et al. 2013). The literature has also detected inter-individual differences not only in responses to pain but also in responses to intervention (Fillingim 2005).

In summary, the findings of no overall effects of hypertonic saline infusion on RMS EMG activity are difficult to explain by earlier models. The possibility of individual effects are possibly more in accordance with more recent models that propose that motor behaviour is variable between different individuals and effects are mediated at multiple levels of the nervous system and these effects may depending the experience or perception of pain (Hodges and Tucker 2011; Murray and Peck 2007). One of these more recent theories also suggests that individual pain-related effects result 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 and Peck 2007).

5.5 Single motor units from temporalis

To our knowledge, this is the first characterization of the effects of noxious stimulation of one jaw muscle on the activity of SMUs in a non-painful synergistic jaw muscle during a standardized jaw closing task. Table 4-27 and table 4-32 summarize the occurrence of each SMU from the right temporalis muscle under the ramp jaw closing tasks and 2 step-levels jaw closing task respectively. From these tables, a summary has been constructed and is shown in Table 5-1 which lists the percentages of SMUs that exhibited some change in the occurrence (recruitment and/or de-recruitment) of SMU activity during each of the tasks or did not change.

	Ramps	Step 1	Step 2
Changed	40/75 (54%)	15/37 (40%)	12/58 (21%)
Did not change	35/75 (46%)	15/37 (40%)	32/58 (55%)

Table 5-1: For the 75 SMUs discriminated for the ramp jaw closing tasks, 40 SMUs (54%) exhibited some change in their occurrence and the remaining 35 SMUs (46%) did not change their occurrence during the tasks and between blocks. From the 37 SMUs discriminated for the step 1 of the 2 step-levels jaw closing task, 15 SMUs (40%) did not change their occurrence, and 15 SMUs (40%) did exhibit some change in their SMU occurrence, and in 7 SMUs it was not possible to confirm if they were present in all of the blocks due to a technical issue. From the 58 SMUs discriminated for the second step of the 2 step-levels jaw closing task, 32 SMUs (55%) did not change the occurrence, 14 SMUs (21%) did exhibit

some change in their occurrence and 12 presented a technical issue that was not possible to state if their presence were confirmed during the 4 blocks or not.

Also, by comparing specifically the hypertonic saline infusion with the isotonic saline infusion, table 5-2 summarizes the number of units and the percentage that were recruited only for the hypertonic saline infusion, that were de-recruited for the hypertonic saline infusion or that were present in both conditions (hypertonic and isotonic saline infusion):

	I x H SR	I x H FR	I x H step 1	I x H step 2
No change	53/70 *(75.7%)*	53/71 *(74.6%)*	27/37 *(73%)*	48/58 *(82.8%)*
Recruited	12/70 (17.1%)	9/71 (12.7%)	6/37 (16.2%)	6/58 (10.3%)
De-recruited	5/70 (7.2%)	9/71 (12.7%)	4/37 (10.8%)	4/58 (6.9%)

Table 5-2: Number of units and the percentage that were recruited only for the hypertonic saline infusion, that were de-recruited for the hypertonic saline infusion or that were present in both conditions. I: isotonic saline infusion; H: hypertonic saline infusion; SR: slow ramp jaw closing task; FR: fast ramp jaw closing task; Step 1: step 1 of the 2 step-levels jaw closing task; step 2: step 2 of the 2 step-levels jaw closing task.

These present findings extend the findings from analogous studies in the lower limb and the jaw motor system. Proportionally, the number of units that were identified during both conditions (pain and no pain) in the present study, that is, their occurrences

were unaffected by pain, were higher than in a previous study (Tucker et al. 2009) which discriminated a total of 52 units in the quadriceps muscle and 34 in the flexor pollicis longus muscle (FPL) during low-force contractions with pain (hypertonic) and without pain (baseline). Of these units, only 20 (38.5%) in the quadriceps and 9 (26.5%) in the FPL units were identified during both trials while all the remaining units discharged only with or without pain, but not in both conditions. But the present study is indeed in accordance with other recent studies where the majority of the SMUs collected from the masseter were present in both conditions (pain and no pain) (Malik 2016; Minami et al. 2013). This apparent difference between the earlier limb studies and the recent (Malik 2016; Minami et al. 2013) and present studies in the jaw, might be explained by methodological differences between the studies given the different motor systems being studied. The difference between studies may also reflect possible differences in the central control systems driving single motor units between masseter and limb and trunk muscles. For example, in comparison with the jaw motor system, the limb motor system may exhibit greater flexibility in recruitment patterns for a specific task.

To our knowledge there have been no studies with intramuscular electrodes about the effects of noxious jaw muscle stimulation on SMU activity in non-painful synergistic jaw muscles. However, there have been many studies of possible effects on non-painful synergistic jaw muscles (and other muscles groups) obtained from surface EMG electrodes (Ciubotariu et al. 2004; Gizzi et al. 2015; Kumar et al. 2015b; Malik 2016; Sae-Lee et al. 2008a; Sandoval 2017; Schulte et al. 2004; Svensson and Arendt-Nielsen 1995). Some of these earlier studies have shown effects of noxious jaw muscle stimulation on the EMG activity of non-painful synergistic muscles (Ciubotariu et al.

2004; Gizzi et al. 2015; Sae-Lee et al. 2008b) while other studies do not show effects (Hodges et al. 2008; Kumar et al. 2015b). For example, in a recent study (Kumar et al. 2015b), experimental pain was induced in the left masseter and left TMJ with a 3-4 day interval between pain sessions. Analysing the surface EMG activity, the left masseter pain did not evoke a significantly different activity in the muscles analysed (left masseter, right masseter, left temporalis, digastric) during maximum voluntary bite force. However, the pain induced in the left TMJ resulted in significantly different EMG activity of the anterior temporalis and anterior digastric muscles. The findings of the present study shows that no effect was noted on the RMS EMG activity (see Tables 4-25 and 4-26) of the temporalis muscle after noxious hypertonic saline injections into the masseter muscle, which is consistent with the earlier findings of no EMG effects on the surface EMG activity.

However, other studies have shown effects on synergistic and antagonistic non-painful muscles (Kumar et al. 2015a; Sae-Lee et al. 2008b). For example, in a standardized jaw opening movement performed during hypertonic saline infusion into the right masseter muscle in comparison with isotonic saline infusion, not only was the surface EMG activity of the right masseter significantly affected but also the surface EMG activity of the left masseter, the right digastric and right inferior head of the lateral pterygoid muscle (Sae-Lee et al. 2008b). The presence of significant EMG effects noted in the jaw muscles in these earlier studies and the absence noted in the RMS EMG activity in the present study may relate to task differences. Thus, the study of Sae-Lee et al employed jaw opening and lateral and protrusive jaw movement tasks that involved tracking a target; there was no added resistance to the jaw movements in these tasks. The present study was an isometric jaw closing task demanding higher

force generation. It is possible that jaw muscle EMG activity at the level of an RMS or surface EMG analysis may not be affected by jaw muscle noxious stimulation where significant closing forces (e.g. 50-150 N) are required but may be more subject to changes in global EMG activity for light jaw movements as was demonstrated in the earlier study (Sae-Lee et al. 2008b). The analysis of SMU activity in the present study showed that most SMUs were unaffected in their occurrences during hypertonic saline vs. isotonic saline infusion. A SMU analysis has not been done for the studies of effects of noxious jaw muscle stimulation on the jaw muscle activity during light jaw movements as employed in the previous study (Sae-Lee et al. 2008b). However, this is an avenue for further investigation.

5.5.1 Carry-over or possible persistent effect of pain; fatigue

Despite the presence of pain in this study, a few factors should be considered for these pain experimental models. For example, there may be possible carry-over effects of the pain induced by the hypertonic saline infusion on subsequent pain-free recordings. The possibility of muscle fatigue may also be a factor, and the performance of the participant on executing a specific task.

In terms of possible carry-over effects, SMUs 81, 82 and 83, for example, were present for slow and fast ramps during the hypertonic and baseline 2 blocks (see Table 4-32). For those units, the solution applied first was the isotonic saline, and the hypertonic saline infusion was done just before the baseline 2 block. One possibility therefore is that the hypertonic saline solution could have had a carry-over effect on the

subsequent baseline 2 block, and which manifest as the presence of those units in this block as well. As another example, SMUs 3, 21 and 22 were not present in the baseline (block 1), but were at least present in all the other infusion blocks (i.e. blocks 2 and 3) (Table 4-32). This pattern of recruitment could also reflect a carry-over effect of the solution applied first (hypertonic) and which may have resulted in a SMU remaining active in the next recording session of isotonic infusion if it became initially recruited in the previous hypertonic saline session.

It is possible therefore, that carry-over effects could be a factor in the pattern of occurrence of SMU activity in the jaw motor system in the present study. If so, then the data suggest that, even though noxious jaw muscle stimulation resulting in pain may provide the trigger for rapid neuroplastic changes in the brain (manifesting as changes in recruitment patterns of SMUs), the removal of the noxious stimulation (and associated pain) may not be enough to result in an immediate return to the original motor output. The factors that trigger a return to normal motor output following the resolution of acute muscle pain are unknown (Schabrun et al. 2017). However, the evidence in the present study for possible carry over effects is not consistent with a recent study that showed that a bolus injection of hypertonic saline into the masseter muscle in healthy individuals does not lead to post-pain changes in jaw movement or jaw-muscle activity during chewing that are caused by the previous experience of pain (Inamoto et al. 2017). This study did not find any significant main effects of group (pain infusion, control) on jaw movement and jaw-muscle activity during the opening and closing phases of chewing after resolution of pain, although the authors recommended other experimental designs examining possible persistent effects of pain on motor activity. Another factor to consider is the possible influence of practice or training

effects with repetitions of the task, which could also have played a role in the present study. Practice or training effects were minimised by alternating the sequence of infusion between participants.

Nonetheless, there is evidence in the literature that changes in muscle activity associated with experimental or clinical low-back pain or knee pain can persist beyond the period of the pain, and this evidence suggests a possible carry-over effect (Hides et al. 1996; Hodges et al. 2003; MacDonald et al. 2009; Moseley and Hodges 2005; 2006; Tucker et al. 2012; Tucker and Hodges 2009). For example, in an earlier study (Tucker and Hodges 2009), changes in SMU recruitment patterns were noted during experimental pain in comparison with control and these effects persisted even after the pain had subsided.

In the jaw motor system, previous rat and human studies have shown prolonged decreases in face primary motor cortex (face M1) excitability that outlasted the duration of the stimulus following noxious jaw or tongue muscle stimulation (Adachi et al. 2008; Nash et al. 2010). Recently the neuroplastic capabilities of the face SI and face MI have been reviewed (Avivi-Arber et al. 2011). This neuroplasticity allows for functional adaptation (or maladaptation) of the orofacial sensorimotor system to an altered oral state or oral motor behaviour and it is likely that longer term changes occur in the face MI during noxious stimulation of the orofacial area. Analogous findings of changes in motor cortex excitability have been reported in limb motor cortex studies (Farina et al. 2001; Le Pera et al. 2001).

Another factor to consider in the interpretation of these SMU data is the possibility of fatigue as an explanation for changes in SMU activity patterns between blocks. This factor was partly controlled by alternating the sequence with which the hypertonic saline and isotonic saline solutions were administered between successive participants. In addition, only 3 trials were performed for each task and the tasks were mostly at low force levels in relation to the maximum forces that most people can perform (Kumar et al. 2015b; Takaki et al. 2014). Therefore, it is considered that fatigue is unlikely to be a major factor in the patterns of recruitment of SMUs. Nonetheless, some of the data could be interpreted in terms of the occurrence of fatigue in some participants in the present study. For example, SMUs 57 and 59 were present in all the blocks but were not present in the second baseline.

Fatigue is a common symptom in chronic pain disorders and there are strong positive correlations between fatigue levels and pain intensity (Boggero et al. 2014; Boggero et al. 2017; de Leeuw et al. 2005) and exhausting muscle contraction is also found to induce change in its EMG activity. For example, a recent study involved a 20-minute tooth-clenching task (50% of maximal voluntary contraction force) and analysed pain (Numeric rating scale 0-10) and fatigue (Borg's Ratings of Perceived Exertion 6-20) throughout microdialysis. They concluded that tooth-clenching increased jaw muscle pain and fatigue (Louca Jounger et al. 2017). However, the duration and magnitude of clenching in this study was much greater than in the present study where the total time involved in exerted bite forces was around 7 minutes.

5.5.2 SMU activity and the vicious cycle theory and the pain adaptation model

An analysis was done unit by unit from the temporalis muscle to see if the data supported or not the earlier models (namely, the Vicious Cycle Theory and Pain Adaptation Model). The summary table (Table 4-41) show that there was only little evidence for support for both of these earlier models. These findings are consistent with an extensive 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).

In fact, from the 83 units discriminated from the temporalis muscle in this study, the majority of the units (56/70 for the slow ramp jaw closing task, 53/71 for the fast ramp jaw closing task, 27/37 for the step 1 of the 2 step-levels jaw closing task and lastly, 48/58 for the step 2 of the 2 step levels jaw closing task) were neither consistent with the Vicious Cycle Theory nor the Pain Adaptation Model. This analysis was based on the presence or not of a SMU during task performance for a comparison of hypertonic saline infusion with isotonic saline infusion. As indicated above, this analysis has a limitation that it does not take into account possible changes in firing rates of SMUs, which could support or not one of the earlier theories.

The Pain Adaptation Model proposes that during pain, agonist muscle activity is reduced in the generation of forces in an attempt to minimize the pain and this inhibition operates at the brainstem level by inhibiting reflex circuits and/or by modifying the central pattern generator for mastication (Lund et al. 1991). The present data show

that these possible inhibitory effects on α-motoneuronal activity from nociceptive activity were insufficient to prevent the descending drive, likely to be from the face area of the primary motor cortex, from activating and recruiting many of the SMUs required during the three tasks developed in this study (slow ramp, fast ramp and 2 step-levels jaw closing task). The goal-directed nature of the tasks therefore was able to reverse, or minimize the effects of, any possible inhibitory effects in the brainstem as proposed by the Pain Adaptation Model.

The finding of a small number of SMUs that was present during the isotonic saline infusion but was not during the hypertonic saline infusion, is indeed consistent with the Pain Adaptation Model. This finding is consistent with other recently reported studies where noxious masseter or tongue muscle stimulation results in inhibitory influences at the level of the primary motor cortex (Adachi et al. 2008; Nash et al. 2010) and where inhibitory effects on agonist jaw muscle activity have been noted with noxious jaw muscle stimulation (Sae-Lee et al. 2008b).

The ability to continue to perform the ramp and step tasks during the hypertonic saline infusion even with the cessation of SMU activity can possibly mean that other SMUs are recruited either within the masseter or in other jaw closing muscles, and this has been demonstrated in the present study for the temporalis muscle. Potentiation of twitch force is another possibility for maintenance of force and it 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 van Eijden 2000).

The number of neural strategies possible to perform a task might decrease as the force magnitude increases as more and more units necessarily become recruited. It is possible that the force levels used in the present study, and/or the nature of the biting task may have been why most units were unaffected by the noxious stimulation. In terms of the nature of the specific biting tasks performed in the present study, it is possible that a specific pattern of recruitment of SMUs is best suited and therefore possibly less likely to be modifiable by an external intervention, e.g. a noxious stimulus. Most of the findings (in terms of occurrences) did not appear to be consistent with the pain adaptation model and this was noted for both of the muscles analysed (masseter and temporalis). This suggests that the ability to override any inhibitory effects on SMU activity during tasks is a generalized feature throughout all agonist muscles involved in jaw tasks. Clearly, more studies are needed to confirm this tentative assumption.

In the case of the Vicious Cycle Theory, the present findings showed that the occurrence of most of the SMUs recorded in the temporalis muscle during the different jaw tasks exhibited a pattern that was not consistent with a critical component of the vicious cycle theory that pain leads to increased jaw muscle activity. This finding is consistent with the findings from a number of previous studies where in general, the vicious cycle hypothesis was not supported (Graven-Nielsen and Arendt-Nielsen 2008) – for reviews see (Murray and Peck 2007; Murray et al. 2014).

The small number of SMUs that was not present during the isotonic saline infusion but that was recruited during the hypertonic saline infusion, provide a pattern of activity

that was indeed consistent with the Vicious Cycle Theory. This finding of recruitment of SMUs in the presence of noxious stimulation is consistent with some other studies. For example, the activation of the sternocleidomastoid during a multidirectional isometric task in chronic neck pain patients found increased muscle activity across all movement directions, thus, supporting the vicious cycle theory (Falla et al. 2010). In addition, evidence of increased agonist and antagonist muscle activity has been reported with hypertonic saline induced experimental masseter muscle pain during some standardized jaw movement tasks (Sae-Lee et al. 2008b).

Therefore, rather than supporting these earlier models that propose uniform increases or reductions in muscle activity during pain throughout a muscle, the new data show that both recruitments and de-recruitments of SMU activity can occur in the agonist muscles of the present study and that these effects likely involve all the muscles involved in task performance. While these earlier models did not imply restriction of pain-related motor effects to just the muscle in pain, these earlier models appear to assume that uniform effects do occur throughout a muscle. The data of the present study do not support this view but rather 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 (Hodges and Tucker 2011; Murray and Peck 2007).

5.5.3 Thresholds of SMU activation

The analysis of the mean threshold values of the SMUs recorded from the temporalis muscle for the slow and fast ramp jaw closing tasks during all recording blocks was also performed for testing for any interactions between the infusion blocks. There were no significant differences between blocks. This is consistent with a few studies where the thresholds did not appear to change due to pain (Malik 2016; Sohn et al. 2000). In both these studies, SMUs were recorded from the masseter muscle during infusion of hypertonic saline or capsaicin into the same muscle and during performance of a biting task. There were no effects noted on SMU thresholds from the masseter during these tasks under pain in comparison with control. Variation in the threshold of firing of SMUs during pain is an additional mechanism to maintain force output despite the de-recruitment of other SMUs. Thus, for example, a reduction in threshold of a particular SMU would mean that force would be generated earlier from that SMU, and this might compensate for the loss of activity of other SMUs.

A possible explanation for the finding that the recruitment threshold of the SMUs did not seem to decrease during painful contraction in the present study, could be that the recruitment of additional SMUs was sufficient to maintain constant force output without changing the recruitment threshold. Changes in firing rate of some existing SMUs might also contribute; however, there was no evidence of a change in firing rate of SMUs during hypertonic saline infusion in comparison with isotonic infusion (see section 5.5.4). Another possibility is that individual SMUs might be differentially affected in terms of threshold by the nociceptive stimulus. This was not analysed in the present study but could be an avenue of further investigation that would involve repetition of more trials of a task so that statistical analysis could be carried out on individual SMUs.

There was a significant difference in repeating the ramp tasks for one or more of the blocks. While this was only noted for a few of such analyses, it may relate to some greater variability in task performance under certain conditions. This was not analysed in the present thesis but is a possible avenue for further research. Alternatively, a person has a broad range of neural strategies for performing one specific task, and it might be different based on many factors, such as the presence of pain, the task performed, how motivated the person was to perform that task or due to neuromuscular adaptations related to learning optimal muscle activation patterns. Changes in neural strategies may also have contributed to this variability in repeating the tasks.

5.5.4 Firing rates

A qualitative analysis of the effects of hypertonic saline infusion, in comparison with isotonic saline infusion, on firing rates was carried out and of these 20 SMUs that were studied in this way at step 1 of the 2 step-levels jaw closing task, 8 single motor units showed a decrease in firing rates, and 6 single motor units showed an increase in firing rates when comparing hypertonic vs. isotonic recording sessions. Also, 4 single motor units “3, 27, 63, 76” were absent during the hypertonic block while 2 single motor units “5, 69” were absent for the isotonic block. For the 15 SMUs studied at step 2 of the 2 step-levels jaw closing task, 3 single motor units showed a decrease in firing rates, and 9 single motor units showed an increase in firing rates when comparing hypertonic vs. isotonic recording sessions. The remaining units were de-recruited in one of the blocks.

These possible effects may simply reflect variation in firing rates activity related to, for example, small variations in the force levels achieved in the step tasks. Alternatively, the present analysis suggested that these individual differences in firing rates activity between hypertonic saline infusion and isotonic saline infusion actually reflect an individual pain-related effect and was not simply due to the fact that the participant exerted more or less force in a particular block.

For the step 1, from the 8 SMUs that decreased their firing rates activity during hypertonic saline infusion, 2 presented lower force during the hypertonic saline block and 6 presented lower force during the isotonic saline block. On the remaining 6 SMUs, that increased their firing rates activity during the hypertonic saline infusion, 2 presented lower force during the hypertonic saline infusion and 4 presented lower force during the isotonic saline infusion.

For the step 2, from the 3 SMUs that decreased their firing rates activity during hypertonic saline infusion, all 3 presented lower force during the isotonic saline block. On the remaining 9 SMUs, that increased their firing rates activity during the hypertonic saline infusion, 2 presented lower force during the hypertonic saline infusion and 7 presented lower force during the isotonic saline infusion.

Overall there was no significant difference in firing rates of SMUs when comparing the hypertonic and the isotonic saline infusion blocks. This finding is consistent with previous findings (Malik 2016; Minami et al. 2013) who found no significant difference in firing rates of SMUs between pain (hypertonic) and no pain (Isotonic) sessions in the masseter muscle during the performance of a biting task. However some SMUs

discriminated from this more recent study (Malik, 2016) showed increases (4 at right masseter inferior/right masseter posterior and 8 at RMS/RMA) and decreases (5 at RMI/RMP and 3 at right masseter superior/right masseter anterior) in firing rates during the hypertonic infusion session in comparison to the isotonic infusion session. These changes in firing rates observed at a SMU level may reflect actual pain-related changes in activity and as such are in agreement with the current study which also found increases and decreases in firing rates.

One possible explanation for the absence of an overall effect of pain on the firing rates is that the same force is achieved by the recruitment of additional higher threshold motor units during the painful contraction while lower threshold motor units are de-recruited (or vice-versa). This reorganization could also explain why surface EMG amplitude remained constant despite the pain in this study.

This explanation does not appear to be consistent with the findings of a study that shows that changes in firing rates of low threshold motor units in muscles with a synergistic function to the painful muscle do not appear to account for the maintenance of force during a painful constant force contraction as motor unit firing rate was reduced in synergist muscles (Hodges et al. 2008). This also suggests the effect of nociceptor stimulation is not localized and has a broad effect on synergist muscles (Hodges et al. 2008).

5.5.5 Sequence of recruitment

The data from 12 participants were able to be analysed for an analysis regarding the sequence of recruitment of SMUs during the slow ramp and fast ramp jaw closing tasks based on the mean thresholds.

For the comparison of hypertonic saline infusion and isotonic saline infusion during the slow ramp jaw closing tasks, there were 12 participants where the sequence of recruitment could be established. Of these 12, 8 participants exhibited recruitment sequences that remained the same under both blocks, and 4 participants changed their recruitment sequence. Interestingly, from the 8 sequence orders obtained that did not change, the isotonic saline solution was applied first in 5 of them, and for the remaining 3 hypertonic saline was applied first. However, for the 4 participants where the order of recruitment did change, the first solution applied was the hypertonic saline.

For the comparison of hypertonic saline infusion and isotonic saline infusion during the fast ramp jaw closing tasks, there were 12 participants where the sequence of recruitment could be established. Of these 12, 7 participants exhibited recruitment sequences that remained the same under both blocks, and 5 participants changed their recruitment sequence. Interestingly, from the 7 sequence orders obtained that did not change, the isotonic saline solution was applied first in 3 of them, and for the remaining 4 hypertonic saline was applied first. However, for the 5 participants where the order of recruitment did change, the hypertonic saline solution was applied first in 4 of them, and only 1 had isotonic saline solution applied first.

These data from both tasks suggest that there may be an effect on the sequence of recruitment simply from the order in which the solutions are applied. When hypertonic saline is applied first, then the data is suggestive that it may be more likely for there to be a change in recruitment sequence. It is unclear the reason for this other than possibly some psychological effects related to the first experience of an infusion.

Changes in the recruitment order have been previously reported for the masseter muscle in association with hypertonic saline infusion (Malik 2016) and has also been reported in the limb literature (Falla et al. 2008; Madeleine et al. 2006; Samani et al. 2009; Tucker and Hodges 2009). A study of neck muscle activity in pain (Stephenson and Maluf 2010) also indicate that changes in recruitment and de-recruitment order in trapezius motor units can occur during short- as well as long-duration contractions.

All of this together suggested that pain interacts in a unique way in the individual with the complex and individualized organization of the sensorimotor system and pain experience (Hodges and Tucker 2011; Mogil 1999; Murray and Peck 2007; Raber and Devor 2002; Sae-Lee et al. 2008a; Sae-Lee et al. 2008b; Wiesinger et al. 2013).

5.6 Single motor units from masseter

A re-organization of EMG activity of the masseter muscle after the injection of hypertonic saline, with increases and decreases occurring within the painful muscle has also been demonstrated in a previous study although with a simpler set of tasks

(Malik 2016; Minami et al. 2013). Therefore, even though it was not our main question in this study, the recruitment of SMUs from the right masseter was analysed during baseline, hypertonic and isotonic blocks of infusion to confirm or not the earlier findings and to establish comparative data for the findings from the temporalis muscle as both were recorded simultaneously.

5.6.1 Occurrence

Table 4-52 and table 4-57 summarize each SMU occurrence from the right masseter muscle under ramps jaw closing tasks and 2 step levels jaw closing task respectively. Three block conditions (baseline, hypertonic infusion and isotonic infusion) were analysed.

Five units were present only during hypertonic blocks for slow and fast ramps but were not present during baseline or isotonic blocks. Another 5 SMUs were not present for slow and fast ramps during hypertonic but were present during baselines and isotonic blocks for slow and fast ramps tasks. Units 34 and 35 were not present in hypertonic and isotonic blocks of infusion but were present for baseline block; while unit 27 was present in hypertonic and isotonic block of infusion but not present for baseline block. Unit 5 was present only during hypertonic fast ramp but was not present in the other blocks for slow ramp. Unit 1, on the other hand, were not present during hypertonic fast ramp but it was present in slow ramp all the blocks and for the remained blocks in fast ramp.

These patterns of change in activity are comparable to previous reports from the masseter muscle (Malik 2016). The present findings extend these earlier findings by carrying out the experiments in another group of participants as well as studying different rates of ramp tasks. The findings provide evidence that in all tasks, a reorganization of SMU activity with recruitments and de-recruitments can occur within the masseter muscle during experimental pain.

5.6.2 SMU activity and the vicious cycle theory and the pain adaptation model

An analysis was also done unit by unit from the masseter muscle to see if the data supported or not the earlier models (vicious cycle theory and pain adaptation model). As was established for the temporalis muscle data, there was only little evidence for support for both of these earlier models.

In fact, from the 58 units discriminated from the masseter muscle in this study, the majority of the units (33/47 for the slow ramp jaw closing task, 37/49 for the fast ramp jaw closing task, 23/28 for the first step of the 2 step-levels jaw closing task and lastly, 33/35 for the second step of the 2 step levels jaw closing task) were neither consistent with the Vicious Cycle Theory nor the Pain Adaptation Model. Again, together with the temporalis muscle, the findings obtained from the masseter muscle in this present study did not provide strong support for either of the earlier theories. Rather the

evidence supported the ideas of a reorganization of motor unit activity within the jaw muscles in experimental muscle pain.

5.7 Comparison between the masseter and temporalis muscles occurrence and consistence with VCT and PAM – as an important comparison

According to th e tables 4-65, 4-66, 4-67, the percentages of SMUs that did not change occurrence, or were recruited or de-recruited during the hypertonic saline infusion block were similar between masseter and temporalis muscles for all the tasks analysed. This suggests that a reorganization of the activity occurs in a proportionally similar way in the painful muscle as well as in synergistic muscles. This could be considered unexpected in that it might be presumed that the painful muscle might undergo a greater amount of reorganization than any of its synergists.

Regarding the consistency with the earlier theories, according to the table 4-68, the percentages of units that did or not did not support either the Vicious Cycle Theory or the Pain Adaptation Model were similar for all the tasks within a muscle. However, there were possibly some differences between muscles, in that the SMUs from the temporalis supported the VCT with a relatively higher percentage in step 1 (16.2%) and step 2 (10.3%) of the 2 step-levels jaw closing task, than the masseter whose percentages were 7.1% in step 1 and 0% in step 2. Furthermore, the masseter supported the PAM with a relatively higher percentage (17%) in the slow ramp jaw closing task than the corresponding values for the temporalis at 7.1%. The reason for this difference is unclear although it might suggest that, in comparison with the painful muscle, the SMUs within the non-painful synergistic muscle were more likely to

undergo changes in activity (i.e. recruitment during pain) that would lend stronger support for the VCT during the 2 levels of the 2 step-levels jaw closing task. The SMUs of the painful muscle appeared possibly more likely to undergo a change in activity (i.e. de-recruitment) that would provide support for the PAM during the slow ramp jaw closing task. Although about 75% of SMUs in both muscles were unaffected in their occurrence in pain vs control, the above observations possibly suggest that pain may be more likely to inhibit the muscle activity of the painful muscle and increase the activity of the non-painful synergistic muscle at least in the set of tasks performed in the present study. These preliminary observations are an avenue of further research.

5.8 Psychological variables

A novel observation in the present study was the preliminary data suggesting that those participants who did not exhibit a change in recruitment patterns (i.e. no evidence of recruitment of new SMUs nor de-recruitment of SMUs) during hypertonic saline infusion in comparison with isotonic saline infusion, were found to exhibit significantly higher PCS scores than those participants who did exhibit a change in recruitment patterns (i.e. evidence of recruitment and/or de-recruitment of SMUs) (see Tables 4-69, 4-70, 4-71, 4-72). Given the small sample size, further studies are necessary to determine whether indeed this is a consistent observation in higher pain catastrophizing individuals. A recent study found a significant, positive correlation between PCS scores and the change in EMG activity of the left temporalis muscle (r = 0.541; P = 0.031) and right temporalis muscle (r = 0.531; P = 0.034) in free chewing at the closing phase after injection of hypertonic saline (Inamoto et al. 2017).

One interpretation of the absence of evidence for recruitments/de-recruitments of SMUs in pain in higher pain catastrophizing individuals is that these individuals may exhibit a loss of fine control or subtle modulation of motor unit activity during pain. The individuals exhibiting evidence of recruitments and de-recruitments during pain were those with lower pain catastrophizing scores. These individuals may exhibit a greater ability to modulate SMU activity in the presence of pain.

A recent study has shown that experimental pain in asymptomatic individuals with higher pain catastrophizing scores is not only associated with increased VAS pain scores, pain mapping areas and McGill pain rating indices than lower pain catastrophizing individuals but that the higher pain catastrophizing individuals also exhibit slower jaw velocity and greater variability of repetitive jaw movements than the lower pain catastrophizing individuals (Akhter et al. 2014). These repetitive jaw movements carried out in this earlier study are likely driven largely from the primary motor cortex (face MI) - for review, (Avivi-Arber and Sessle 2017). Noxious jaw muscle stimulation is known to have significant effects on face MI activity with brief increases in signal intensity followed by prolonged decreases (Nash et al. 2010) and these effects are consistent in general terms with observations in the rat with noxious stimulation of the tongue (Adachi et al. 2008) and in limb muscle studies (Farina et al. 2005; Tucker and Hodges 2009). Further, there is recent evidence that the changes in MI activity with noxious jaw muscle stimulation correlated with pain catastrophizing scores (Henderson et al. 2016). While it is not clear whether the motor mechanisms involved in higher pain catastrophizers in the present study are in some way different from those in lower pain catastrophizers, one interpretation of the present data is that the ability

of the face MI to exert fine control over SMU recruitment patterns may become impaired in higher pain catastrophizing individuals in pain. This might indeed account for a greater variability of jaw movements as previously noted in simulated chewing movements in higher pain catastrophizers in pain (Akhter et al. 2014). This may also account therefore for the inability to modify or modulate motor unit activity in pain as noted in the present study for SMU recruitment patterns.

5.9 More complex models of pain-motor interaction

The findings of the present study support the ideas of the Integrated Pain Adaptation Model which proposes that the experience of pain (e.g., acute/chronic, localization - muscle/joint, prior experience, beliefs, emotional contributions, motivation, social context, genetic) will affect motor activity differently in each person. A recent clinical study of chronic TMD patients, specifically those with severe symptomatology, provided evidence for a reorganized activity, mainly resulting in worse functional performances (Mapelli et al. 2016), which is in also in accordance with a new model that proposes a new theory to explain the adaptation to pain (Hodges and Tucker 2011).

Therefore, the authors that first proposed this model also suggested that all chronic pain patients should not be treated exactly the same way, even when they have the same physical diagnosis (Sae-Lee et al. 2008b), and this approach of treating everyone differently has previously demonstrated successful outcomes (Dworkin et al. 2002).

6 CONCLUSIONS

The relation between pain and muscle activity is not clearly understood and earlier models explaining this association, namely the Vicious Cycle theory and the Pain Adaptation Model, are not strongly supported by the literature. While both theories propose uniform increases or decreases in activity throughout a painful muscle, recent evidence in both the spinal and trigeminal literature suggests that there are likely to be complex changes of activity within a painful muscle indicating a re-organization of activity. It is unclear however whether this reorganization of activity within a painful jaw muscle also occurs in other non-painful jaw muscles.

Another issue with these earlier theories is that they do not take psychological factors into consideration and yet psychological factors are known to be important in the onset and progression of TMD. It is not known whether psychological factors might influence the reorganization that appears to be occurring within the jaw muscles during pain.

The present study has employed EMG recordings of single motor unit activity from the masseter and temporalis muscles during experimentally induced jaw muscle pain to assess whether changes in SMU activity (e.g. recruitments and de-recruitments of SMUs) can occur not only within the painful masseter muscle but also in the non-painful temporalis muscle. Associations between the changes in activity and some psychological variables were also analysed.

The experimental infusion paradigm of hypertonic saline employed in the masseter muscle for our study did not affect the ability to perform the biting tasks employed (slow ramp jaw closing task, fast ramp jaw closing task, 2 step-levels jaw closing task) in terms of force amplitudes and force rates in comparison with control infusions of isotonic saline. In addition, there was no group effect on the root mean square EMG activity from the masseter and temporalis muscles, major agonists of the tasks. Furthermore, in comparison with isotonic saline infusion, there was no effect of the hypertonic saline infusion into the masseter on the occurrences of most of the SMUs within the masseter and temporalis muscles during the tasks, as well as no group effect on temporalis SMU thresholds during the ramp tasks and no group effect on temporalis SMU firing rates during the step tasks. However, during hypertonic saline infusion in comparison with isotonic saline infusion, evidence was provided for both recruitment and de-recruitment of SMU activity for about 50% of SMUs within the masseter and for about 54.2% of SMUs within the temporalis muscle. The changes in occurrence of SMUs in general did not support the Vicious Cycle Theory or the Pain Adaptation Model. However, where changes in occurrence of SMUs did occur, a qualitative observation was that the changes within the temporalis muscle, the non-painful synergistic muscle, were more likely to undergo recruitments during pain in some of the tasks, while the SMUs of the painful masseter muscle appeared possibly more likely to undergo a de-recruitment of SMU activity at least in the slow ramp task. Finally, suggestive evidence was provided for associations between SMU occurrence in pain and PCS scores in that those participants who did not exhibit a change in recruitment patterns (i.e. no evidence of recruitment of new SMUs nor de-recruitment of SMUs) during hypertonic saline infusion in comparison with isotonic saline infusion, were

found to exhibit significantly higher PCS scores than those participants who did exhibit a change in recruitment patterns.

Taken together, these findings suggest that in the presence of noxious stimulation of one jaw muscle, reorganization of SMU activity occurs throughout the jaw motor system during task performance and it might be suggest that non-painful muscles should be treated in TMD patients to help the entire jaw motor system undergo a reorganization back to normal function. Preliminary data suggests that the changes may be different in the painful vs. non-painful synergistic muscles and the effects may be influenced by the level of pain catastrophizing. These new data are more in line with more recent models of pain-motor interaction (Hodges and Tucker 2011; Murray and Peck 2007) than the earlier Vicious Cycle Theory or the Pain Adaptation Model. This new information may help to improve our understanding of the effects of pain on jaw muscle activity and thereby may have implications for understanding changes in jaw muscle activity in TMD.

7 LIMITATIONS:

7.1 Differences between short-term experimental pain and chronic pain patients

There are differences between short-term experimental pain and chronic pain patients that limit the conclusions that can be drawn from this study for the chronic pain population. However, while there are differences between human experimental pain models and chronic pain patients, the findings from human experimental pain models can, at least in part, help us to understand clinical pain states as the pain evoked shares many clinical features with those seen in chronic clinical muscle pain.

It is important to mention, however, that there are studies in the literature that show similarities between experimental pain models and clinical pain patients. For example, a recent study (Louca et al. 2014) investigated if hypertonic saline-induced myalgia also results in the same levels of biomarkers as found in the muscles of chronic myalgia patients. They observed that 5-HT, glutamate and glycerol levels increased after the saline injections and similar increases have been reported in chronic myalgia patients (Ernberg et al. 1999; Gerdle et al. 2008). Furthermore, some of the McGill Pain Questionnaire pain descriptors reported for experimental jaw muscle pain (Sae-Lee et al. 2008b) are the same as those identified in another study (Gustin et al. 2011) which evaluated chronic pain patients with Temporomandibular Disorders. The presence of referred pain is also common in experimental pain (Jensen and Norup 1992) as well as chronic conditions (McMahon et al. 1995). Finally, experimentally induced nociception from back muscles, or the resulting pain perception, appears able to

change the control of trunk muscles in a manner resembling that observed in clinical low back pain (Hodges and Moseley 2003; van Dieen et al. 2003).

Therefore while there are differences between human experimental pain models and chronic pain patients, experimental pain models may be able, at least in part, to help us to understand clinical pain states as the pain evoked shares many clinical features with those seen in chronic clinical muscle pain. And it is hoped that these studies will generate important baseline information and hypotheses for future clinical studies.

7.2 Isotonic saline infusion associated with some levels of pain

This study compared experimentally induced pain with pain-free blocks and isotonic saline was used for the pain-free block so that the same conditions existed between both blocks, and the only difference between the blocks was pain. However, the isotonic saline did induce some pain in some participants, which might be caused by the discomfort of the needle and the expansion of the muscle due to the volume of the isotonic saline and therefore in some participants it was not possible to be considered as totally free pain condition.

7.3 Small sample

Also, due to the small sample size, it is difficult to generalize some of the findings of this study and further studies should be performed on a larger number of volunteers.

This would also assist in providing additional data supporting or not some of the preliminary conclusions.

7.4 Intramuscular electrodes on antagonist side

Despite the fact that the present study has allowed us to accurately observe the effect of experimental right masseter muscle pain on single motor unit activity at the right masseter and right temporalis muscles, we did not study the effect of pain on SMU activity within contralateral muscles.

7.5 Multiple recording

Another limitation of the present study is that we recorded SMUs from only one site in each of the masseter and temporalis muscles in each participant, and therefore, we limit our conclusions to one area of the muscle and our data cannot provide an overall assessment across different compartments of the muscles. Also, multi-channel surface EMG obtained over large regions of the masseter and temporalis muscles would be a useful future investigative tool to map the global changes in EMG activity across the muscles in association with noxious stimulation.

7.6 Baseline 2

In the current study baseline 2 was only analyzed for RMS activity from masseter and from temporalis, and for SMU occurrence from the temporalis and comparison between blocks within the temporalis muscle. However, it was not analyzed for force amplitude values; force rates values; force levels values; thresholds and firing rates of the SMUs from the temporalis; SMUs occurrence from the masseter muscle and comparison between blocks within the masseter muscle. This is a possible avenue for future studies. Also, in this study the 2 infusions ran immediately next to each other and were only separated by 10 min. Future studies could consider having another control block of trials between both infusion blocks. This could be used to establish a new baseline for the subsequent infusion block.

7.7 Maximal voluntary biting force

No information on maximal voluntary biting force was obtained in this study. However, based on the reference values found by Kowaga et al., 2006 for maximum bite force, we calculated that the force levels in the present study were approximately 13% of the maximum bite force on the slow ramp, 15% on the fast ramp, 10% on step 1 and 12% on the step 2 of the jaw closing tasks. Nonetheless, it is important to mention that maximum bite force levels vary with method, sex, age, anatomical and physiologic characteristics of the subjects and therefore, precise information on maximal voluntary biting force should be obtained in further studies (Kogawa et al., 2006).

7.8 Use of upper and lower splints leading to a bite-raise

The use of the splints devices in the upper and lower jaw will have had caused a bite-raise that resulted in the jaw being opened an estimated ~8mm. This increase in the bite might have had implications for the SMU activity as during the rest period prior to the tasks, the participant might have had affected baseline activity simply due to the fact that the jaw was opened. However, it is important to reinforce that the analysis was performed within participants, i.e. the same amount of increase of the bite was used during all the blocks of tasks for the same participant. Therefore, the presence of possible activity that was possibly due to the bite raise, did not represent a problem in the interpretation of differences of SMU activity during hypertonic stimulation in comparison with the other blocks analysed in this study.

7.9 Changes in the infusion rates

The changes in the infusion rates throughout the infusion blocks were not recorded in this study. As a suggestion for future studies, this information may be valuable as it would allow the researcher to analyse if the different task or different forces applied would require a higher or a lower rate of infusion. This may provide information, therefore, as to possible differences between tasks of the perceived pain.

7.10 Psychological findings

The relationship between the psychological variables with the SMU findings are intriguing but are only based on very few participants and can only be viewed as

preliminary data. We would like to suggest further investigation including also some subjects with high PCS scores to enable testing for possible correlations of SMU patterns in individuals covering a wide range of PCS scores.

8 Appendix

8.1 Clinical Research Diagnostic Criteria (RDC) Examination/ RDC/TMD history questionnaire

CLINICAL RESEARCH DIAGNOSTIC CRITERIA (RDC) EXAMINATION

1. Do you have pain on the right side of your face, the left side or both.

None	0
Right	1
Left	2
Both	3

2. Could you point to the areas where you feel pain?

	Right		Left
None	0	None	0
Jaw Joint	1	Jaw Joint	1
Muscles	2	Muscles	2
Both	3	Both	3

(Examiner feels area subject points to, if it is unclear whether it is joint or muscle.)

3. Opening Pattern

Straight	0
Right Lateral Deviation (uncorrected)	1
Right Corrected (“S”) Deviation	2
Left Lateral Deviation (uncorrected)	3
Left Corrected (“S”) Deviation	4
Other	5
Type(specify) _____	

4. Vertical Range of Motion — Maxillary incisor used

(0)	1.1
(1)	2.1

(a) Unassisted opening without pain _____ mm

	MUSCLE PAIN				JOINT PAIN			
	None	Right	Left	Both	None	Right	Left	Both
(b) Maximum unassisted opening _____ mm	0	1	2	3	0	1	2	3
(c) Maximum assisted opening _____ mm	0	1	2	3	0	1	2	3

(d) Vertical incisal overlap _____ mm

CLINICAL RESEARCH DIAGOSTIC CRITERIA (RDC) EXAMINATION

5. Joint Sounds (palpation)		RIGHT	LEFT
(a) Opening	None	0	0
	Click	1	1
	Coarse Crepitus	5	5
	Fine Crepitus	6	6
Measurement of Opening Click		_____ mm	_____ mm
(b) Closing	None	0	0
	Click	1	1
	Coarse Crepitus	5	5
	Fine Crepitus	6	6
Measurement of Closing Click		_____ mm	_____ mm

(c) Reciprocal click eliminated on protrusive opening

	RIGHT	LEFT
	No	No
	Yes	Yes

6. Excursions		MUSCLE PAIN				JOINT PAIN			
		None	Right	Left	Both	None	Right	Left	Both
(a) Right Lateral Excursion	_____ mm	0	1	2	3	0	1	2	3
(b) Left Lateral Excursion	_____ mm	0	1	2	3	0	1	2	3
(c) Protrusion	_____ mm	0	1	2	3	0	1	2	3

(d) Midline Deviation _____ mm (0) RIGHT (1) LEFT (2) NA

CLINICAL RESEARCH DIAGOSTIC CRITERIA (RDC) EXAMINATION

7. Joint Sounds on Excursions

Right Sounds:	None	Click	Coarse Crepitus	Fine Crepitus
Excursion Right	0	1	5	3
Excursion Left	0	1	5	3
Protrusion	0	1	5	3

Left Sounds:	None	Click	Coarse Crepitus	Fine Crepitus
Excursion Right	0	1	5	6
Excursion Left	0	1	5	6
Protrusion	0	1	5	6

DIRECTIONS FOR ITEMS 8-10

The examiner will be palpating (touching) different areas of your face, head and neck. We would like you to indicate if you do not feel pain of just feel pressure (0), or pain (1-3). Please rate how much pain you feel for each of the palpations according to the scale below. Circle the number that corresponds to the amount of pain you feel. We would like you to make a separate rating for both the right and left palpations.

0 = No Pain/ Pressure Only
1 = Mild Pain
2 = Moderate Pain
3 = Severe Pain

PLEASE GIVE PATIENT RATING CARD

CLINICAL RESEARCH DIAGOSTIC CRITERIA (RDC) EXAMINATION

8. Extraoral muscle pain with palpation:

	RIGHT	LEFT
a. Temporalis (posterior) "Back of temple"	0 1 2 3	0 1 2 3
b. Temporalis (middle) "Middle of temple"	0 1 2 3	0 1 2 3
c. Temporalis (anterior) "Front of temple"	0 1 2 3	0 1 2 3
d. Masseter (superior) "Cheek/under cheekbone"	0 1 2 3	0 1 2 3
e. Masseter (middle) "Cheek/side of face"	0 1 2 3	0 1 2 3
f. Masseter (inferior) "Cheek/jawline	0 1 2 3	0 1 2 3
g. Posterior mandibular region (Stylohyoid/posterior digastric region) "Jaw/throat region"	0 1 2 3	0 1 2 3
h. Submandibular region (Medial pterygoid/Suprahyoid/anterior digastric region) "Under chin"	0 1 2 3	0 1 2 3
i. Sternocleidomastoid (origin) "Under ear"	0 1 2 3	0 1 2 3
j. Sternocleidomastoid (body) "Side of neck"	0 1 2 3	0 1 2 3
k. Trapezius (origin) "Back of head"	0 1 2 3	0 1 2 3
l. Trapezius (body and insertion) "Neck and shoulders	0 1 2 3	0 1 2 3

9. Joint pain with palpation:

a. Lateral pole "Outside"	0 1 2 3	0 1 2 3
b. Posterior attachment "Inside ear"	0 1 2 3	0 1 2 3

10. Intraoral muscle pain with palpation:

a. Lateral pterygoid area "Behind upper molars"	0 1 2 3	0 1 2 3
b. Tendon of temporalis "Tendon"	0 1 2 3	0 1 2 3
c. Tongue "Tongue"	0 1 2 3	0 1 2 3

11. Is pain on same side as reported in question 2? Yes No

RDC/TMD HISTORY QUESTIONNAIRE

Please read each question and respond accordingly. For each of the questions below circle only one response.

1. Would you say your health in general is:

Excellent Very good Good Fair Poor

2. Would you say your oral health in general is:

Excellent Very good Good Fair Poor

3. Have you had pain in the face, jaw, temple, in front of the ear or in the ear in the past month? No

Yes

[If no pain in the past month go to question 14]

If Yes,

4 a. How many years ago did your facial pain begin for the first time? _____years ago

b. How many months ago did your facial pain begin for the first time? _____months ago

5. Is your facial pain persistent, recurrent or was it only a one-time problem? Persistent

Recurrent

One-Time

6. Have you ever gone to a physician, dentist, chiropractor or other health professional for facial ache or pain? No

Yes, in the last six months

Yes, more than six months ago

7. How would you rate your facial pain on a 0 to 10 scale at the present time, that is right now, where 0 is "no pain" and 10 is "pain as bad as could be"?

NO PAIN PAIN AS BAD AS COULD BE

0 1 2 3 4 5 6 7 8 9 10

8. In the past six months, how intense was your worst pain rated on a 0 to 10 scale where 0 is

"no pain" and 10 is "pain as bad as could be"?

NO PAIN PAIN AS BAD AS COULD BE

0 1 2 3 4 5 6 7 8 9 10

9. In the past six months, on the average, how intense was your pain rated on a 0 to 10 scale where 0 is "no pain" and 10 is "pain as bad as could be"? [That is, your usual pain at times you were experiencing pain].

NO PAIN PAIN AS BAD AS COULD BE

RDC/TMD HISTORY QUESTIONNAIRE

10. About how many (e.g. 60) days in the last six months have you been kept from your usual activities (work, school or housework) because of facial pain? _____DAYS

11. In the past six months, how much has facial pain interfered with your daily activities rated on a 0 to 10 scale where 0 is "no interference" and 10 is "unable to carry on any activities"?

NO INTERFERENCE ON ANY ACTIVITIES — UNABLE TO CARRY

0 1 2 3 4 5 6 7 8 9 10

12. In the past six months, how much has facial pain changed your ability to take part in recreational, social and family activities where 0 is "no change" and 10 is "extreme change"?

NO CHANGE — EXTREME CHANGE

0 1 2 3 4 5 6 7 8 9 10

13. In the past six months, how much has facial pain changed your ability to work including housework) where 0 is "no change" and 10 is "extreme change"?

NO CHANGE — EXTREME CHANGE

0 1 2 3 4 5 6 7 8 9 10

14 a. Have you ever had your jaw lock or catch so that it won't open all the way?

No

Yes

If Yes, b. Was this limitation in jaw opening severe enough to interfere with your ability to eat?

No

Yes

15 a. Does your jaw click or pop when you open or close your mouth or when chewing?

No

Yes

b. Does your jaw make a grating or grinding noise when it opens and closes or when chewing? No

Yes

c. Have you been told, or do you notice that you grind your teeth or clench your jaw while sleeping at night?

No

Yes

d. During the day, do you grind your teeth or clench your jaw?

No

Yes

f. Do you have noises or ringing in your ears? No

Yes

g. Does your bite feel uncomfortable or unusual?

No

Yes

16 a. Do you have rheumatoid arthritis, lupus, or other systemic arthritic disease?

No

Yes

b. Do you know of anyone in your family who has had any of these diseases?

No

Yes

c. Have you had or do you have any swollen or painful joints) other than the joints close to your ears (TMJ)? No

Yes

If Yes, d. Is this a persistent pain which you have had for at least one year? No

Yes

17 a. Have you had a recent injury to your face or jaw?
No

Yes

If Yes,

b. Did you have jaw pain before the injury? No

Yes

18. During the last six months have you had a problem with headaches or migraines?

No

Yes

19. What activities does your present jaw problem prevent or limit you from doing?

a. Chewing	No Yes	g. Sexual activity	No Yes
b. Drinking	No Yes	h. Cleaning teeth or face	No Yes
c. Exercising	No Yes	i. Yawning	No Yes
d. Eating hard foods	No Yes	j. Swallowing	No Yes
e. Eating soft foods	No Yes	k. Talking	No Yes
f. Smiling/laughing	No Yes	I. Having your usual facial appearance	No Yes

21. How good a job do you feel you are doing in taking care of your health overall?

Excellent

Very good

Good

Fair

Poor

22. How good a job do you feel you are doing in taking care of your oral health?

Excellent

Very good

Good

Fair

Poor

23. When were you born? Day_____Month_____Year_____

24. Are you male or female? Male

Female

25. In what country were you born? _____

26 a. Does this country best represent your race, national origin or ancestry?

Yes

No

If No,

b. What is your country of national origin or ancestry? _____

27. What is the highest grade or year of regular school that you have completed?

Never attended or Kindergarten

Primary School

High School

University

28 a. During the past 2 weeks, did you work at a job or business not counting work around the house (include unpaid work in the family farm/business)?

Yes

No

N/A

If No, b. Even though you did not work during the past 2 weeks, did you have a job or business?

Yes

No

N/A

If No,

c. Were you looking for work or on layoff from a job during those 2 weeks?

Yes, looking for work

Yes, layoff

Yes, both on layoff and looking for work

No

N/A

29. Are you married, widowed, divorced, separated or never been married?

Married / spouse or defacto in household

Married / spouse or defacto not in household

Widowed

Divorced

Separated

Never Married

N/A

8.2 Screening questionnaire

SCREENING QUESTIONNAIRE

Please indicate whether you have used any of the following in the past 24 hours:

	(circle one)	(if YES)
CAFFEINE (e.g. tea/coffee)	YES / NO	When _____ Qty _____
ALCOHOL	YES / NO	When _____ Qty _____
MEDICATION	YES / NO	

If YES, please specify:_____

Have you ever received treatment for TMD and/or orofacial pain (e.g. trigeminal neuropathic pain)?

YES / NO (circle one) If YES, please specify: _____

Have you ever had TMD and/or orofacial pain?

YES / NO (circle one) If YES, please specify: _____

Have you ever received treatment for other pain disorders?

YES / NO (circle one) If YES, please specify: _____

Are you pregnant or expecting for pregnancy?

YES / NO (circle one) If YES, please specify: _____

Are you currently taking any medication prescribed by a doctor?

YES / NO (circle one) If YES, please specify: _____

Have you ever received treatment for any of the following:

	(circle one)	(If YES – give details please)
HEART PROBLEMS	YES / NO	_____
RESPIRATORY PROBLEMS	YES / NO	_____
DIABETES	YES / NO	_____
BLOOD PRESSURE	YES / NO	_____
BLEEDING DISORDERS	YES / NO	_____
COMMUNICABLE DISEASES (e.g. Hepatitis)	YES / NO	_____
NEUROLOGICAL DISORDERS (e.g. Parkinson's)	YES / NO	_____
PSYCHIATIC DISORDERS (e.g. Depression)	YES / NO	_____
RHEUMATIC FEVER	YES / NO	_____
SYSTEMIC DISORDERS (e.g. chronic malignancies)	YES / NO	_____

Do you have any medical conditions that the current researchers should be made aware of?

YES / NO If YES, please specify: _____

(circle one)

Do you suffer from migraine YES / NO

Do you suffer from sinusitis?	YES / NO
Have you had full body or cranial CT scans in the last 12 months?	YES / NO
Do you have a medical condition where it is anticipated that CT scans may be required?	YES / NO
Do you have any heart pacemaker, defibrillator or wires other than sternal wires?	YES / NO
Do you have metallic foreign body in the eye?	YES / NO
Do you have deep brain stimulator, cerebral aneurysm clips or cochlear implant?	YES / NO
Do you have prosthetic implants or dentures?	YES / NO
Do you feel claustrophobic when you are in a confined, noisy environment?	YES / NO

Name of Participant _____

Signature _____ *Date* _____

8.3 DASS 21

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 (e.g. 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 I 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 (e.g., 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

8.4 PCS

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.	

8.5 McGill Questionnaire

MC GILL PAIN QUESTIONNAIRE (MPQ)
Melzack, 1985.

Some of the words below may describe your **PRESENT** pain. Circle **ONLY** those words that best describe your pain at this moment.

1	Flicking Quivering Pulsing Throbbing Beating Pounding	2	Jumping Flashing Shooting	3	Pricking Boring Drilling Stabbbing Lancinating	4	Sharp Cutting Lacerating
5	Pinching Pressing Gnawing Cramping Crushing	6	Tugging Pulling Wrenching	7	Hot Burning Scalding Searing	8	Tingling Itchy Smarting Stinging
9	Dull Sore Hurting Aching Heavy	10	Tender Taut Rasping Splitting	11	Tiring Exhausting	12	Sickening Suffocating
13	Fearful Frightful Terrifying	14	Punishing Grueling Cruel Vicious Killing	15	Wretched Blinding	16	Annoying Troublesome Miserable Intense Unbearable
17	Spreading Radiating Penetrating Piercing	18	Tight Numb Drawing Squeezing Tearing	19	Cool Cold Freezing	20	Nagging Nauseating Agonising Dreadful Torturing

8.6 McGill Questionnaire – analysis

Category	Group	Score / Words
Sensory	1	1 Flicking
		2 Quivering
		3 Pulsing
		4 Throbbing
		5 Beating
		6 Pounding
	2	1 Jumping
		2 Flashing
		3 Shooting
	3	1 Pricking
		2 Boring
		3 Drilling
		4 Stabbing
		5 Lancinating
	4	1 sharp
		2 cutting
		3 Lacerating
	5	1 Pinching
		2 Pressing
		3 Gnawing
		4 Cramping
		5 Crushing
	6	1 Tugging
		2 Pulling
		3 Wrenching
	7	1 Hot
		2 Burning
		3 Scalding
		4 Searing

	8	1 Tingling
		2 Itchy
		3 Smarting
		4 Stinging
	9	1 Dull
		2 Sore
		3 Hurting
		4 Aching
		5 Heavy
	10	1 Tender
		2 Taut
		3 Rasping
		4 Splitting
Affective	11	1 Tiring
		2 exhausting
	12	1 Sickening
		2 Suffocating
	13	1 Fearful
		2 Frightful
		3 Terrifying
	14	1 Punishing
		2 Grueling
		3 Cruel
		4 Vicious
		5 Killing
	15	1 Wretched
		2 Blinding
Evaluative	16	1 Annoying
		2 Troublesome
		3 Miserable
		4 Intense
		5 Unbearable
Miscellaneous	17	1 Spreading
		2 Radiating
		3 Penetrating

		4 Piercing
	18	1 Tight
		2 Numb
		3 Drawing
		4 Squeezing
		5 Tearing
	19	1 Cool
		2 Cold
		3 Freezing
	20	1 Nagging
		2 Nauseating
		3 Agonising
		4 Dreadful
		5 Torturing

Table 8-1: Pain Rating Index –PRI and words values in ascending order.

2 -13

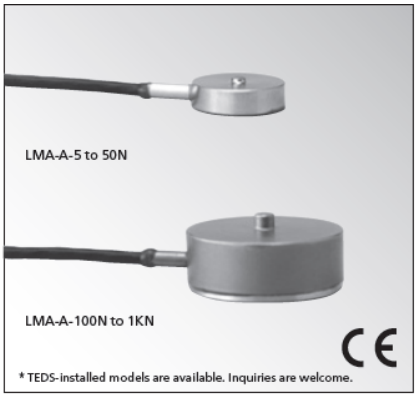
TRANSDUCERS

Load Cells (Load Transducers)

LMA-A

●ϕ12 mm ●Thickness: 4 mm (5N to 50N) ●5 N to 1 kN

Small-sized Compression Load Cell



* TEDS-installed models are available. Inquiries are welcome.

Compact & Lightweight Moderate Price Suitable for Load Distribution Measurement

Compact and lightweight LMA-A series load cells are used by just putting or bonding on the measurement point or setting in a hollow.

Specifications

Performance

Rated Capacity	See table below.
Nonlinearity	Within ±1% RO
Hysteresis	Within ±1% RO
Repeatability	1% RO or less
Rated Output	0.6 to 2 mV/V for 5 N
	0.75 to 2 mV/V for 10 N to 1 kN

Environmental Characteristics

Safe Temperature	-10 to 60°C (Non-condensing)
Compensated Temperature	0 to 50°C (Non-condensing)
Temperature Effect on Zero	
	Within ±0.3% RO/°C for 5 N
	Within ±0.2% RO/°C for 10 to 50 N
	Within ±0.05% RO/°C for 100 N to 1 kN
Temperature Effect on Output	
	Within ±0.2%/°C for 5 to 50 N
	Within ±0.05%/°C for 100 N to 1 kN

Electrical Characteristics

Safe Excitation	7 V AC or DC
Recommended Excitation	1 to 5 VAC or DC
Input Resistance	350 Ω±2.5%
Output Resistance	350 Ω±2.5%
Cable	4-conductor (0.035 mm²) vinyl shielded cable, 1.7 mm diameter by 2 m long, bared at the tip (Shield wire is not connected to the case.)

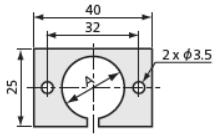
Mechanical Properties

Safe Overloads	150%
Natural Frequencies	See table below.
Weight	5 to 50 N: Approx. 1.5 g, excluding cable
	100 N to 1 kN: Approx. 11 g, excluding cable
Material	5 to 50 N: Copper alloy
	100 N to 1 kN: Stainless steel
Degree of Protection	IP64 (IEC 60529)
RoHS Directive	EN50581

Optional Accessories: Mount Base CFM-A

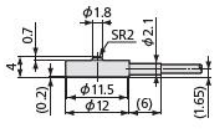
Models	Rated Capacity	Natural Frequencies (Approx.)
LMA-A-5N	5 N	15.3 kHz
LMA-A-10N	10 N	17.5 kHz
LMA-A-20N	20 N	24.8 kHz
LMA-A-50N	50 N	32.6 kHz
LMA-A-100N	100 N	21.6 kHz
LMA-A-200N	200 N	29.7 kHz
LMA-A-500N	500 N	43.9 kHz
LMA-A-1KN	1 kN	53.0 kHz

Mount Base CFM-A

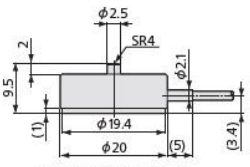


Models	A	Thickness	Remarks
CFM-5A	12.2	1.5	For 5 to 50 N
CFM-100A	20.2	3.0	For 100 N to 1 kN

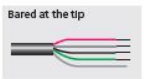
Dimensions



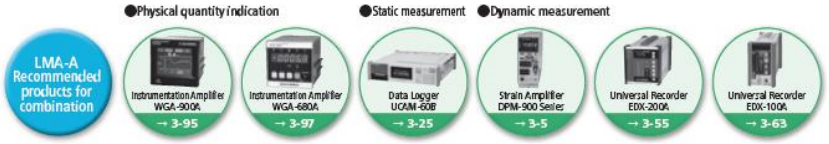
LMA-A-5N to 50N



LMA-A-100N to 1KN



●Physical quantity indication ●Static measurement ●Dynamic measurement



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