MECHANICAL MONITORING OF INHIBITORY JAW REFLEXES IN HEALTH AND SIMULATED DYSFUNCTION

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I, Mounir Atassi, hereby declare that I am the author of this thesis and that all references cited have been consulted by myself. I was the principal investigator in all studies described in this thesis. This work has not previously been submitted for a higher degree in this or any other university.

Mounir Atassi
Certificate

I hereby certify that Mounir Atassi has fulfilled the conditions of Ordinance 39 of the University of Dundee and is qualified to submit this thesis for the Doctor of Philosophy.

Professor Samuel W. Cadden
Abstract

Objectives: Previous studies in the Oral Neurophysiology Laboratories in Dundee have defined the electromyographic properties of the inhibitory jaw reflex that can be evoked in human subjects by electrical stimulation of the lip. This reflex, in contrast with the more widely studied biphasic inhibitory reflexes evoked by stimulation of intra-oral nerves, consists of just a single phase of inhibition and usually requires the application of stimuli which excite nociceptive nerves. The aims of the present studies were to define the mechanical manifestations of this reflex in the form of changes in biting forces, and to investigate whether the mechanical manifestation of the inhibitory jaw reflex evoked by stimulation of the human upper lip, can be modulated by experimentally-controlled conditions that mimic symptoms of a myogenous temporomandibular disorder.

Methods: Three series of experiments were performed on 49 volunteer subjects in total. The experiments involved recording bite forces between the anterior teeth and electromyograms (EMGs) from the masseter muscles. Transcutaneous electrical stimuli were applied to the hairy skin of upper lip while the subjects maintained a biting force of around 50N with the aid of visual feedback. In the first series of experiments, a range of electrical stimuli below and above the nociceptive threshold was delivered. In the second set of experiments, double stimuli with a range of different inter-stimulus intervals were applied. Finally in a third series of experiments, electrical stimulation was repeated before, immediately after, and 5 and 10 minutes following a 3-minute accelerated chewing task. This task consisted of chewing 1.5g
of a tough chewing gum at 1.5 times the subject’s natural chewing rate and in 18 cases, muscle fatigue and/or pain were reported by the subjects.

**Results:** Following stimulation at intensities that were described as sharp or painful, all the subjects showed both a suppression of the masseter EMG and a reduction of biting force. When analysing the maximum responses in each subject, the mean reduction in the EMG inhibition was to 15.78 ± 14.4% and 10.39 ± 7.92% of the baseline (for the ipsi- and contra-lateral EMGs respectively), whereas the biting force was reduced only to 83.98 ± 11.04% of baseline (+ S.D.). The latencies of onset of these responses were: 38.17 ± 3.58ms, 38.97 ± 4.49ms and 51.83 ± 6.23ms respectively. The response observed in the force record was weaker than in that observed in either EMG (Paired t tests, P < 0.005 in both cases). When applying double stimuli, it was found that the prolongation of the EMG inhibitory jaw reflex (to 144.70 ± 46.93% of the control level) evoked by double stimulation of the upper lip (with a 10 ms inter-stimulus interval) resulted in a greater increase in the depth of the accompanied relaxation (to 223.63 ± 70.88% of that seen in the control responses) compared to a relatively smaller increase in the duration of the relaxation (to 128.32 ± 27.23% of that seen in the control responses). Following the accelerated chewing task, 17 out of 22 subjects reported pain and/or fatigue in one or both of the masseter muscles. The integral for the bite force relaxation significantly decreased in size immediately following the conditioning procedure (to 76.04 ± 35.63% of the control level, P = 0.014; single sample t-test with Bonferroni correction, test value 100).
Conclusion: The inhibitory jaw reflex evoked by stimulation of the human lip can be demonstrated mechanically as well as electromyographically although the mechanical version of the response appears less marked. In addition to that, the onset of reflex relaxation in bite force lags several milliseconds behind the corresponding reductions in electromyographic activity. The depth of force relaxation can be increased by increasing the duration of EMG recorded inhibitory reflex. Finally, the results from a chewing task suggest that induced acute pain and/or fatigue cause clear changes in the mechanical manifestation of this inhibitory jaw reflex.
Chapter 1: Introduction

1.1 General Introduction

The masticatory apparatus exerts very complicated interactions during different functional activities. The jaw muscles are known to be controlled by three different classes of movement: voluntary, complex integrated activities (or involuntary generated patterns) and reflexes. The reflexes in the jaw muscles are believed to play a major role in the modulation of the other two inputs (i.e. voluntary and complex integrated activities). A phasic reflex can be specified as an instantaneous, transient and predictable response to a given stimulus. A typical reflex arc consists of receptor(s), an afferent neuron, interneuron(s) and an efferent neuron. Therefore, studies of jaw reflexes are often viewed as providing insights into the synaptic relationship of afferent systems to motor neurons within the trigeminal system (Orchardson and Cadden, 1998). Reflexes in the masticatory muscles can be elicited by stimuli in or around the mouth. There are three primary types of vertical reflexes that can be invoked in the jaw muscles: i.e. jaw jerk, jaw unloading and inhibitory jaw reflexes. In this work, it was the inhibitory jaw reflexes that were under study, and thus this section will focus on this set of reflexes in particular.

In addition to being useful to investigate the physiology of the masticatory organs, the studies of reflexes in the masticatory muscles might also provide better understanding of the pathophysiology of the organs. Jaw reflexes have been the subjects of considerable clinical research since the suggestion by Bessette et al. (1971) that the
duration of the silent period following a jaw jerk elicited by chin tap may have diagnostic value.

Until now, jaw reflexes have been studied almost exclusively by making electromyographic (EMG) recordings - usually from one muscle. However, some of the jaw muscles are not easily accessible to make EMG recordings and there is a lack of information on how various reflex effects on the jaw muscles are related to the overall loading forces between the jaws. Without this information, it is not possible to predict how much one jaw muscle (usually masseter) may represent the overall changes in bite force in response to muscles activity during jaw reflexes. It is believed that bite force recording (in adjunct to EMGs) can provide valuable data to enhance our understanding of the pathological changes in jaw reflexes that is seen in clinical conditions such as temporomandibular disorders (TMD) (e.g. De Laat et al., 1985).

In this chapter, the origins, the underlying mechanisms and the modulation of inhibitory jaw reflexes will be discussed. Additionally, the use of bite force recordings in the study of the inhibitory reflexes will be reviewed.
1.2 Inhibitory Jaw Reflexes

The existence of an inhibitory reflex in the jaw closing muscles has been known since the time of Sir Charles Sherrington (1917). This reflex is believed to be analogous to the withdraw reflex in the limbs. In all animals, a noxious stimulus in or around the mouth leads to a reduction in the activity of the jaw closing muscles (Yemm, 1972b). In contrary to what is seen in animal studies (e.g. cats or rats) the inhibitory reflex is not accompanied with activation of the opening muscles in primates – including human beings (for review see Orchardson and Cadden, 1998). Although, this reflex is sometimes referred to as “jaw-opening reflex” in human studies, such terminology is more appropriate to animal research in the light of the nature of the reflex in animals.

The existence of the jaw-opening reflex in humans is a controversial subject. Yemm (1972a) and Matthews (1976a) reported no digastric or infrahyoid muscle activation accompanying the inhibitory jaw reflex evoked by noxious stimulus to the human oral mucosa. However, it has been found that high intensity stimuli, usually described as unpleasant, can evoke an active jaw opening reflex in man which shows marked habituation (Desmedt and Godaux, 1976; Cadden and Newton, 1988).

The role of inhibitory jaw reflexes is believed to be preventing overloading of the masticatory system, and facilitating jaw opening to expel noxious materials and to minimise any possible damage to intra- or peri-oral structures (De Laat, 1987; Lund, 1991; McKay et al., 1992). In addition to the protective role, the inhibitory jaw reflex may play a significant role in the modulation of jaw movements during mastication (Cadden and Orchardson, 2009).
In response to a given stimulus, the reflex consists of one or two inhibitory periods which may be followed by a period or periods of increased activity. To review further the origin of the inhibitory periods, evidence from studies involving different stimuli and modalities will be discussed in the next section.

1.2.1 Origins of the inhibitory periods

While the jaw muscles are relaxed in man, no recordable response is produced when an electrical stimulus is applied to the oral mucous membrane (Matthews, 1976b). However, the inhibitory reflex can be evoked experimentally using different types of stimulus during voluntary contraction of jaw elevator muscles.

The reflex can be evoked by mechanical stimuli applied to teeth (e.g. Hannam et al., 1969; Louca et al., 1996; Yang and Turker, 1999). Controlled application of tap stimuli results in two inhibitory periods each followed by a period of excitation (van der Glas et al., 1985). The latencies of the inhibitory periods are usually of the order 10 and 40ms respectively, see an example in Figure 1-1. Because the reflex consists of both inhibitory and excitatory components, sometimes it is referred to as the post-stimulus EMG complex (PSEC) (van der Glas et al., 1984a). This response is found to be mediated by the mechanoreceptors in the periodontium (van der Glas et al., 1985; Bonte and van Steenberghe, 1991) and receptors in the inner ear (van Steenberghe et al., 1981). Cadden et al. (1996) reported that the duration of the inhibitory waves increases with increasing the tap stimulus intensity. On the other hand, an application
of a gentle pushing stimulus on a tooth – rather than tooth taping – have been reported to be able to excite low threshold periodontal ligaments receptors and to produce a single short latency inhibitory period (Louca et al., 1996; Scott et al., 2012). Therefore, one could conclude that the long latency inhibitory period, that is seen after tooth taping, may result from stimulating receptors in the adjacent gingival receptors (Louca et al., 1998).

This reflex can also be evoked by normal tooth contact during mastication (Hannam et al., 1969). However, the tooth contact - unlike artificial tooth tapping - results in a single early latency inhibitory period (Ainine et al., 2011).

An alternative way of studying the inhibitory jaw reflex experimentally is by applying electrical stimuli to intra- or peri-oral sites. In most cases, electrical stimulation of virtually any intra-oral structure results in two inhibitory periods in the jaw closing muscles: a short and a long latency inhibitory waves at approximately 13–22 and 40–60 ms respectively (e.g. Yemm, 1972b; Godaux and Desmedt, 1975; Cadden and Newton, 1988; Gardner et al., 2008; Cadden et al., 2013). However, with higher intensities the two inhibitory waves tend to merge together.

In contrast to intra-oral electrical stimulation, electrical stimuli close or above the nociceptive threshold applied to peri-oral structures produces mainly a single long latency inhibitory wave - as seen in Figure 1-1 - at approximately 40 ms (Yu et al., 1973; Cadden and Newton, 1988). Under such circumstances, the short latency inhibitory period – in addition to the long latency inhibitory period - can be evoked only by using very high stimulus intensities (Andersen et al., 1998a). However, this is
believed to be due to stimulus spread to excite intra-oral receptors (Cadden and Newton, 1988).

Although the electrical stimuli are not regarded as natural methods for stimulation – compared to mechanical methods, they have the advantage of being easily controlled and applied. Extra-oral electrical stimuli, on the hair-bearing skin of the lips in particular, provides an easy and well-controlled method for studying inhibitory jaw reflexes and it has been used in our laboratory for a number of studies (e.g. Cadden and Newton, 1988; Cadden et al., 1997; Maillou and Cadden, 1997; 2007; Maillou et al., 2010). As the present study aims at studying the mechanical manifestation of inhibitory jaw reflexes in man, electrical stimulation of the upper lip provided easily evoked and easily-objectively-quantified responses comparing to the more complex (multiphasic) responses seen for intra-orally-evoked reflexes which are more difficult to quantify.

1.2.2 The reflex pathways

The central pathways involved in the inhibitory jaw reflexes in human beings is not entirely clear. The first synapse is thought to be in the supratrigeminal nucleus (SVN) and/or the reticular formation (Figure 1-2). The afferent fibres for the first inhibitory period are believed to first synapse at the level of the midpons (Godaux and Desmedt, 1975; Ongerboer de Visser and Goor, 1976). Interneuron(s) then transmits the impulses to the ipsilateral and contralateral trigeminal motor nuclei. Studying the
inhibitory jaw reflex in patients with brain stem lesions (Ongerboer de Visser et al., 1990) revealed that the afferent fibres for the second inhibitory period follow similar pathway, but impulses are mediated through a polysynaptic pathway, which probably involves the lateral reticular formation at the level of ponto-medullary junction.
Figure 1-1 EMG recordings are showing reflex responses to indicated stimuli. Each record represents three superimposed sweeps of raw masseter EMG. The periods of the inhibitory reflex (periods of relative inactivity) are marked with the red asterisks. The recordings are from previous studies in the Clinical Oral Neurophysiology Research Laboratory at Dundee Dental School (After Cadden and Orchardson, 2009; EMGs courtesy of Dr Andrew Mason).
Figure 1-2 The presumed pathways for the inhibitory jaw reflexes in the peripheral nervous system (PNS) and central nervous system (CNS) in response to intra-oral stimuli. Note that the stimulation of mechanoreceptors and/or nociceptors can trigger two parallel pathways. The short latency inhibitory period is more likely to be mediated through a tri-synaptic pathway (via the supratrigeminal nucleus) while the long latency inhibitory period is more likely to be mediated through a polysynaptic pathway (via the reticular formation). Such a long latency response can also be evoked by stimulation of perioral receptors. After Orchardson and Cadden (1998)
1.2.3 Modulation of inhibitory jaw reflexes in man

Much of the interest in inhibitory jaw reflex studies in the past few decades has been generated from the findings of potentially altered jaw reflexes in patients suffering from TMD. Most of the early studies on the silent period of the jaw jerk reflex in TMD patients (e.g. Bessette et al., 1971; McCall Jr and Hoffer, 1981) has been challenged and doubted due to the lack of methodological rigour and a high intra- and inter-subject variability (van der Glas and van Steenberghe, 1989). However, because the inhibitory reflexes can be examined in a more controlled and objective way, there was an increasing interest in studying these reflexes in TMD patients. A number of groups have examined inhibitory reflexes evoked by intra-oral stimuli (mechanical or electrical) in TMD patients (e.g. Sharav et al., 1982; De Laat et al., 1985; Türker et al., 1989), and reached similar conclusions to each other, namely that there are significant changes in the long latency inhibitory period (mainly a smaller size of reflex). Maillou and Cadden (2007) studied the inhibitory reflex evoked electrical stimuli on the upper lip in TMD patients. Their finding was, again, consistent with previous studies on more complex jaw reflexes evoked by intra-oral stimuli (mentioned above).

The current view of TMD is that it is a cluster of related disorders in the masticatory system and is characterized mainly by pain in jaw muscles and/or the temporomandibular joint (TMJ), and limitation of jaw movement (for review see Svensson and Graven-Nielsen, 2001). It is a widespread clinical problem in modern days. A meta-analysis of 51 studies on TMD occurrence showed a prevalence of 30%
for reported symptoms (Dekanter et al., 1993). The aetiology of TMD is uncertain, but in general it is believed to be multifactorial including occlusal factors, skeletal abnormalities, psychological disturbances and genetic factors (for recent review see Widmer, 2008). The biopsychosocial model (Dworkin and LeResche, 1992) is the most accepted theory to attempt to integrate dynamic and multilevel (physiological, psychological and social) factors at different stages in the development of pain and pain dysfunction (Suvinen et al., 2005). Several hypotheses for the aetiology of TMD have been put forward, challenged and some have been refuted. Amongst these hypotheses is that altered jaw reflexes (i.e. suppressed) could play a role as aetiological factors, as the jaw closing muscles in these patients have a tendency to show increased activity during these reflexes. This would possibly contribute to the persistence of the TMD symptoms. What also could support this hypotheses were the findings that induced stress in asymptomatic subjects (stress is a common symptom in TMD patients) modulate the reflex in a similar way (for review see Cadden, 2007).

In order to understand the possible pathological changes in the jaw reflexes and their cause-effect relationship with the clinical conditions, it was important to know how these reflexes are modulated in physiological conditions.

Much of the work on the modulation of jaw reflexes had focused on mechanisms underlying the modulation of inhibitory jaw reflexes by heterosegmental nociceptive stimuli and/or stress (for review see Cadden, 2007). It has been found that inhibitory jaw reflexes (with different stimulation modalities) are suppressed by heterotopic noxious conditioning stimuli whether the noxious stimuli were of a cutaneous nature
(Mason et al., 2007) or effecting deep somatic structures (Maillou and Cadden, 1997). However, because myogenous pain in jaw muscles is one of the symptoms in TMD patients, it was important to look at the homosegmental conditioning stimuli effect on the inhibitory jaw reflexes. This could provide an insight into the pathological changes in the inhibitory reflexes in TMD patients and therefore be more clinical relevant.

Human experimental pain models are usually used to study pain mechanisms under controlled settings (for recent review see Olesen et al., 2012). These experimentally-induced pain techniques have been employed in the past to study the effect of pain on aspects of somatosensory and motor function in the orofacial region. These experimental methods, to evoke pain under controlled circumstances, could help find answers to the cause-effect relationships, as any subsequent changes in the jaw motor function - in the healthy volunteers - are likely to be as a result of the experimental pain (Lobbezoo et al., 2002). Different homosegmental conditioning stimulation methods have been used to induce pain experimentally in the orofacial region in order to study the effect of pain on the inhibitory jaw reflex in human beings. These methods could be of a cutaneous nature (e.g. thermal) or effecting deep somatic structures (notably the jaw muscles).

Andersen et al. (1998a) found that the application of radiant heat conditioning stimuli on the ipsilateral cheek produces a suppression effect on the late inhibitory wave of the intra-orally induced reflex, whereas negative results were obtained when inducing
a topical spontaneous burning pain sensation to the cheek skin (Kemppainen et al., 1997) or when applying high-intensity pinch stimuli (Biasiotta et al., 2007).

Similarly, studies of noxious homosegmental conditioning of deep somatic structures have produced inconsistent results. Sustained jaw muscle contraction was found to have little or no effect (Maillou and Cadden, 2008), while an accelerated chewing task (van der Kaaij et al., 2009; Maillou et al., 2010) produced significant suppression on the inhibitory jaw reflex in the masseter muscles. More invasive techniques such as hypertonic saline infusion into the masseter muscle have been found to produce a significant effect on the inhibitory jaw reflex (Wang et al., 1999; Svensson et al., 1999).

The lack of significant effect in some of the above studies could be attributed to the fast transient character of the induced nociceptive inputs and/or fatiguing effect in some techniques and/or to the inadequate levels of the induced jaw pain (Lobbezoo et al., 2006).

The suppression of inhibitory jaw reflexes by pain and/or fatigue constitutes a loss of a negative feedback mechanism which usually minimises the risk of overloading structures involved in mastication – this may in turn contribute to the symptoms of TMD (see e.g. Orchardson and Cadden, 1998). However, before making such links, it is important to investigate any potential changes in the mechanical manifestations of the jaw reflexes (i.e. the change in force between the jaws), in addition to monitoring these reflexes by means of electromyographic (EMG) recordings as was done in the previous studies (De Laat et al., 1985; Türker et al., 1989; Maillou et al., 2010). It
would be important to investigate whether the inhibition would show clear signs of producing less unloading of the masticatory apparatus, and not merely less electrical activity in the muscles.
1.3 Mechanical recording of the inhibitory jaw reflexes

Until now, most studies have monitored jaw reflexes by making electromyographic (EMG) recordings from one or more of the jaw muscles. Fewer studies have investigated these reflexes by monitoring the changes in bite force between the teeth in addition to EMG recordings (e.g. Yemm, 1972a; Yamamura et al., 1993; Yang and Turker, 1999; Brinkworth and Türker, 2005). These changes in bite force as a result of the inhibitory reflex may be quantified in terms of latency of onset, duration and “size” (integral) of the response. Yemm (1972a) showed - by using a force transducer - that a transient opening movement takes place in relation to the masseter inhibition evoked by electrical stimulation of oral mucous membrane. Yang and Turker (1999) recorded surface EMG and bite force when applying a mechanical stimulus on an upper incisor tooth. While they observed three different patterns of reflex responses in EMG (i.e. only excitation, only inhibition or inhibition followed by excitation) they observed only a decrease in the net closing force as a result of the same stimulus. They concluded from this finding that the net reflex response of all jaw muscles was best expressed by the averaged bite force recording.

However, none of these studies considered the long latency inhibitory reflex evoked by the stimulation of the upper lip (at a latency of approximately 40-60 ms). As discussed above, this reflex provides an easy and well-controlled method for studying the inhibitory jaw reflex. In addition to that, it is known to have similar characteristics to the longer latency reflex evoked by intra-oral stimuli (Cadden and Newton, 1988) which is believed to be absent or diminished in TMD patients.
1.4 Aims

Facial pain due to various TMD conditions is a significant problem in clinical practice. The question of why patients have such persistent and often severe pain from their jaw muscles and from their temporomandibular joints has still not been answered (for recent review see Lobbezoo et al., 2006). One sensible line of investigation is to study the various reflexes that operate around the jaws as these reflexes have roles in controlling the force of jaw-closing muscles, the co-ordination of jaw-opening and closing muscles and the movement of the temporomandibular joints (for review see Orchardson and Cadden, 1998).

The timing, duration and size of a jaw reflex are usually determined by making electrical (EMG) recordings from one or more of the muscles concerned. Less commonly the reflex may be studied by making mechanical recordings of the resulting changes in force being generated by the muscles. The latter technique has not been employed in any systematic way for jaw reflexes but is necessary to further our understanding of these reflexes. As established by previous studies in Dundee, the site and method of stimulation is important when investigating jaw reflexes (e.g. Cadden and Newton, 1988; Mason et al., 2006). Electrical stimulation on the hair-bearing skin of the upper lip is one appropriate method for evoking the type of reflexes which are thought to be modulated in TMD patients (e.g. De Laat et al., 1985; Maillou and Cadden, 2007). Electrical stimulation is controllable, safe and does not induce unwanted effects such as vibration of nearby tissues.
The aim of this project was to define the parameters of the mechanical manifestations of the jaw reflexes evoked by electrical stimulation on the upper lip and to establish a systematic method to analyse the changes in bite force recordings objectively as a result of the jaw reflex pattern. Once this was accomplished, it was necessary to apply the method and record the reflexes from an asymptomatic group of volunteers before and after an accelerated chewing task. This was undertaken to investigate whether the mechanical manifestation of the inhibitory jaw reflex evoked by stimulation of the upper lip, can be modulated by experimentally-controlled conditions that mimic symptoms of a myogenous TMD.

Therefore, this thesis will now describe series of experiments which were designed to investigate: the mechanical manifestation of the inhibitory jaw reflex that is evoked by electrical stimulation to the upper lip and the effect of experimentally-induced pain and/or fatigue on the mechanically- and electrically-recorded jaw reflex.
2 Chapter 2: Materials and Methods

2.1 General Introduction

This chapter explains the experimental procedures used in the different experiments described in this thesis. All experiments involved the recording of electromyographic (EMG) activities from the masseter muscles and of bite force using a force transducer. Inhibitory jaw reflexes were initiated by the application of electrical test stimuli on the upper lip.

Experiments were performed in the Oral Neurophysiology Research Laboratory, University of Dundee Dental School. The protocols were granted ethical approvals by the institutional ethics committee (University Research Ethics Committee, Ref: 8070) and was in accordance with the guidelines of the Declaration of Helsinki of the World Medical Association (version 2008) (WMA, 2008).
2.2 Subject Selection

Volunteer subjects from postgraduate students and staff groups participated in the experiments. All the participants gave written, informed consent on a detailed verbal and written explanation of the procedures involved.

It was reported previously that patients with myofascial pain dysfunction or other types of oro-facial pains (e.g. toothache) may have altered jaw reflexes (e.g. De Laat et al., 1985). Thus, any subject with a history of craniomandibular dysfunction or current oro-facial pain was excluded from the studies. Because subjects had to bite on a force transducer, they had to be dentate with complete natural anterior teeth.

All subjects volunteered to participate in the studies in accordance with the Ethics Committee guidelines and none of the subjects had a dependent relationship with myself.
2.3 Recording Techniques

2.3.1 EMG recording techniques

The subjects sat comfortably in an upright dental chair and were instructed to clench to aid in locating the main bulk of the masseter muscle by palpation. The skin was cleaned by rubbing with an alcohol wipe (70% isopropyl alcohol) and then allowed to dry. This improves the electrical contact and reduces the impedance between the skin and the electrodes.

EMG activity was recorded using 2 pairs of skin surface electrodes attached over the masseter muscles. In different parts of the project, two different types of electrode were used: (i) In early experiments, silver / silver chloride disc electrodes were used; these were held in place using adhesive discs with 20 mm between the electrodes of each pair. Electrical contact (contact area 19.6 mm$^2$ / electrode) was achieved with a sodium chloride based electro-conductive gel (SIGNAGEL® Electrode Gel). (ii) In later experiments, wet-gel, disposable electrodes were used (Ambu® Neuroline 720). The silver / silver chloride sensors in these electrodes have sensor area of 18 mm$^2$/ electrode. The adhesive area (up to 461 mm$^2$) is bigger than the one in the disc electrodes; therefore, the disposable electrodes were positioned 15 mm apart for each pair. These pre-gelled and self-adhesive electrodes, which are designed for single use, provide enhanced hygiene, patient comfort and reduced application time. The main disadvantages of surface electrodes, comparing to intramuscular techniques (i.e. needle or wire electrodes), are that they cannot be used effectively for recording from deep muscles and that there is potential risk of recording EMG activity from nearby
located muscles "cross-talk" (Basmajian and De Luca, 1985). In the latter case, the 
detection of cross-talk signals from other adjacent muscles could become a concern 
when recording from facial muscles. On the other hand, the detection area for wire 
electrodes may not be representative of the entire muscle and they are difficult to 
apply. For our type of studies, it has been found that the surface-electrode EMG 
technique was the most suitable for assessing inhibitory responses in the masseter 
muscle compared to intra-muscular EMG techniques (Hansen et al., 1998). 

By employing a combination of a head-stage and isolated amplifiers (Neurolog 
NL824 Head stage amplifier and NL820 isolated amplifier, Digitimer, UK), the EMG 
signals were amplified by the order of 10000 and 20000 times. The NL820 amplifier 
had a mute function/plug-in which was triggered in synchrony with the stimulus to 
prevent any artifact as a result of pick-up of the electrical stimulus that was applied on 
the upper lip, not very far from the recording electrodes. In addition, the EMG signals 
were filtered – high pass 20Hz and low pass 1000Hz using a Neurolog NL135 filter 
(Digitimer) – to remove high and low frequency elements which are of no biological 

2.3.2 Bite force recording technique:

Bite force was recorded between the anterior 6 teeth in each jaw (i.e. between all the 
incisors and canine teeth) using a unidirectional force transducer. The force 
transducer consisted of two metal beams with two strain gauges attached to each side
of one beam. The overall thickness of the transducer was 7 mm (Figure 2-1). The transducer was checked for its response time and it was found that this was so rapid that the difference between the start of a controlled externally-applied force and the appearance of this force in the recording was less than 0.5 ms, i.e. the time between successive samples in the digitally converted record (Figure 2-2). The force-transducer signals were amplified and filtered (DC-50Hz) using a bridge amplifier system (NL107 and NL106, Neurolog, Digitimer, Welwyn Garden City, UK). As with the EMG recordings, the force recordings were continuously analogue-to-digital converted (2000 digital points per second), using the intelligent interface connected to the computer running the Signal program (version 2.16; Cambridge Electronic Design, Cambridge, UK).

The use of the bite force transducer is considered to be semi-critical according to Spaulding Classification System (Spaulding, 1968) because it is in contact with intact oral mucosa membrane. Therefore, a high-level of disinfection was required before each experiment. The transducer was wiped with a detergent and soaked in a disinfection solution (10 tablets of Actichlor / 1L water which gives 1% available chlorine) for 2min. The transducer was then washed and wiped with a dry cloth.

The transducer was calibrated before each experiment using a series of weights (2, 5, 10, 15 and 20 Kg) and was found to be linear over a wide range of forces (0 – 196 Newtons). In order to standardize the position of biting throughout each experiment, self-curing chair-side acrylic resin (UnoDent Ltd.) was applied on the biting parts of the metal beams and the subject was asked to bite on the force transducer to make
teeth indexes. This also made the transducer more comfortable for the subject to bite on during the experiment.

Figure 2-1: Photograph of the metal force transducer used in the experiments.

Figure 2-2: Test response of the force transducer.
2.4 Stimulation

In order to evoke reflexes, electrical stimuli were delivered via bipolar skin surface silver / silver chloride disc electrodes of the same type used to record the EMG. These were positioned ~5mm apart, unilaterally just above the vermillion border and lateral to the philtrum. They were held in place with adhesive discs and an electro-conductive gel (SIGNAGEL® Electrode Gel) was used to achieve electrical contact. The stimuli consisted of 1 ms constant-current, rectangular pulses emitted from an isolated constant current stimulator (DS7, Digitimer Ltd.). The stimulator was controlled by a 5V trigger pulse generated by the digital-to-analogue output of the interface (CED 1401 plus, Cambridge Electronic Design, Cambridge, UK) using the Signal program (Figure 2-3). In the first protocol, (see chapter3) the stimuli were delivered in blocks of ten with a 4 seconds interval between individual stimuli. The interval was chosen to prevent the effects of reflex habituation in response to repeated stimuli (Desmedt and Godaux, 1976) and to be able to deliver a reasonable number of stimuli in a reflex block without causing major muscle fatigue which could affect the reflex size/duration (van der Kaaij et al., 2009; Maillou et al., 2010). A mute, starting 1ms before the stimulus and lasting 3ms, was applied to reduce the EMG distortion by the stimulus artifact. The mute was controlled by a trigger block in Signal and delivered to the NL820 amplifier via the digital-to-analogue output of the interface (CED 1401 plus).

Three baseline stimulus levels were determined during the different experiment, sensory threshold, sharpness threshold and reflex threshold. To determine the sensory
threshold, stimuli were triggered manually starting at zero and slowly increasing until the subject reported a sensation, usually a tactile sensation. Then, by further increasing the stimulus intensity until the subject describes the stimulus as sharp. At this sharpness threshold, it is likely that nociceptors are being stimulated. In later experiments, multiples of sensory threshold were used to determine the reflex threshold by analysing the averaged full-wave-rectified records (for details see below).
2.5 Experimental Protocol

During the experiments, the participants were seated upright in a dental chair. In outline, the experiments involved recording bilateral masseter EMG and bite force while the participants bit on a force transducer.

At the start of the experiments, a nociceptive threshold for the electrical stimuli was determined. This was defined as the minimum stimulus required to elicit a sharp sensation. In order to evoke the reflex (see below), a range of multiples of the nociceptive threshold were employed, namely 0.7, 1, 1.4, 1.96, 2.74, 3.84 & 5.38. This algorithmic scale was found to optimize the building of stimulus/response relationship without the need to apply too many intensities. In addition to that, the non-biological EMG noise was recorded at some point during the experiments (before, during or after), by simply asking the subject to fully relax his/her jaw and record quick 10 EMG sweeps. Noise level from the bite force transducer was recorded as any DC shift in the force record by simply recording 10 sweeps without applying any force on the transducer.

The stimuli were applied to the upper lip while the participants produced a controlled level of bite force (around 50N). To this end, the subjects were provided with visual feedback of their force recordings (Figure 2-3). For each stimulation intensity, blocks of 10 (in 6 subjects) or 20 stimuli (in 9 subjects) were delivered in the control experiment. There was no significant difference in all parameters between the two groups (P>0.05, for details see discussions, Chapter 3). Therefore, blocks of 10 stimuli were used in the subsequent experiments.
2.6 Data Capture and Analysis:

The aims of the EMG and bite force analysis were:

- to detect any changes in a baseline of EMG activity following a giving stimulus, in the form of decreases (inhibitory reflex) or excitations;
- to detect the effect of the EMG inhibitions and excitations on bite force recordings;
- to be able to calculate and quantify objectively the deferent parameters of the EMG and bite force reflex responses, in terms of latency of onset, duration and magnitude which reflects both the duration and the size depth/height of the response.

In order to achieve these aims, the EMG signals and force recordings were processed according to the protocol mentioned below.

2.6.1 Data digitization

The EMG signals and bite force recordings were continuously analogue-to-digital converted (2000 digital points per second), using an intelligent interface (CED 1401 plus, Cambridge Electronic Design, Cambridge, UK) connected to a computer running the program Signal (version 2.16; Cambridge Electronic Design, Cambridge, UK) (Figure 2-3). The minimal acceptable sampling rate (at least twice the highest frequency cut-off of the bandpass filter), as specified by Nyquist theorem, was used in
all experiments. The 1401plus provides up to 8 different input (analogue to digital) channels and 3 output (digital to analogue) channels. In my experiments, 6 input channels were used. This includes: 2 channels for raw right and left EMG signals, one channel for bite force recording, one channel for the trigger block and two channels for the full wave rectification of the EMG signals from the first two channels. In every sweep, 2300 ms of signals were captured; 300 ms pre-stimulus and 2000 ms post-stimulus. The reason for the relatively lengthy sweep is to allow for the bite force recording of a slow nature. One of the output channels was used to trigger the stimulator (DS7, Digitimer Ltd.).

Figure 2-3 The general recording and stimulating set-up for the experiments.

*Courtesy of Dr Andrew Mason.*
2.6.2 Data processing

The EMGs were processed by full-wave rectification, averaging and smoothing (Figure 2-4) while the bite force recordings were simply averaged. The smoothing routine for the averaged EMGs involved the application of a Gaussian low-pass filter (70Hz, -3dB). van der Glas and van Steenberghe (1981) concluded that no significant reflex information could be gained from frequencies in excess of 70Hz. After the records had been processed in this way, the average noise level was subtracted and the magnitudes of the EMG recordings were normalised with respect to the mean level of activity in the 200 ms immediately prior to the application of the stimulus (Figure 2-4). As in previous studies in our lab, a custom-made spreadsheet was employed to quantify waves in the processed EMG which represented reflexes (see Gardner et al., 2008; Maillou et al., 2010). The spreadsheet is programmed to select for quantification, the largest inhibitory and excitatory waves around a linear ‘baseline’ (Figure 2-4 and Figure 2-5). Five parameters were quantified for these waves (Figure 2-6): (1) latencies from time of stimulation to onset (ms); (2) durations (ms); (3) latencies from time of stimulation to peak effect (the maximum reduction for inhibitory waves or increase for excitatory waves) (ms); (4) magnitudes of peak effects – expressed as percentages of the baseline activity; (5) integrals with respect to the 100% baseline (%.ms). Exclusion of all waves of shorter duration than 4.7ms, based on the findings of a previous study (Van Der Glas et al., 1995) was used to minimise the risk of labelling chance fluctuations as reflexes.
Similar analyses were performed on the recordings of bite force (Figure 2-5 and Figure 2-6) except that these recordings were simply averaged and quantified using the spreadsheet (as DC recordings they did not require to be rectified and they were not smoothed). The spreadsheet was adjusted and modified to take into account the slow nature of the changes in bite force comparing to the EMG.
Raw Superimposed EMG sweeps

Full wave rectified, superimposed sweeps

Average, normalised, noise subtracted EMG
Figure 2-4: The top two records shows 10 EMG raw sweeps superimposed then full wave rectified – the inhibitory reflex is shown by the * symbol. The bottom record shows the averaged normalised and smoothed record (after subtracting the mean of the noise level). The dotted red horizontal line represents the mean pre-stimulus level. The dashed black vertical line represents the timing of the stimulus.
Figure 2-5: The upper trace shows 10 individual bite force recordings superimposed from the same experiment in Figure 2-4. The second trace shows the same recording after averaging, subtracting the average noise level and normalising to the pre-stimulus level. The dotted red horizontal line represents the mean pre-stimulus level. The dashed black vertical line represents the timing of the stimulus.
Figure 2-6: The top record shows averaged, normalised and smoothed EMG record simultaneous with the bite force record (bottom one). The significant inhibitory and excitatory waves are shaded green and red respectively. The dotted red horizontal line represents the mean pre-stimulus level. The dashed black vertical line represents the timing of the stimulus.
2.6.3 Statistical analysis

Summary data are expressed as means ± s.d. The data were analysed using Repeated Measures Analysis of Variance (RM ANOVA) followed, where appropriate, by Bonferroni-corrected paired t-tests. Chi-squared analysis and independent two sample tests were applied as well. P values of less than 0.05 were regarded as significant. The statistical tests employed will be detailed in each relevant Chapter.
3 Chapter 3: Mechanical Properties of Inhibitory Jaw Reflexes Evoked by Stimulation of the Upper Lip in Man

3.1 Introduction

As discussed in chapter 1, pain due to Temporomandibular Disorders (TMD) can be a major problem in clinical practice. The aetiology of such persistent and often severe pain from jaw muscles and the temporomandibular region has not been fully established. Several hypotheses have been put forward, challenged and some have been refuted. Amongst these hypotheses is that the suggestion that altered jaw reflexes may be aetiological factors (e.g. De Laat et al., 1985; Maillou and Cadden, 2007).

In recent years, the primary focus of research of the Oral Neurophysiology Research Group at Dundee Dental School, has been the physiological variability of inhibitory jaw reflexes (for review see Cadden, 2007). These reflexes have a number of putative roles including minimising the potential overload of the jaw muscles and the TMJ (for review see Orchardson and Cadden, 1998).

Until now, most studies have monitored jaw reflexes by making electromyographic (EMG) recordings from one or more of the jaw muscles. Fewer studies have investigated these reflexes by monitoring the changes in bite force between the teeth in addition to EMG recordings (e.g. Yemm, 1972a; Yang and Turker, 1999). These changes in bite force may be quantified in terms of latency of onset, duration and
“size” (integral or peak value) of the reflex response. However, none of these studies have investigated the long latency (approximately 40-60 ms) inhibitory reflex evoked by the stimulation of the upper lip. This reflex has a similar characteristic to the longer latency reflex evoked by intra-oral stimuli which is believed to be absent or diminished in myogenous TMD patients (Sharav et al., 1982; van der Glas et al., 1984a; De Laat et al., 1985; Türker et al., 1989)
3.2 Aim

The aim of this study was to investigate the mechanical properties of the inhibitory reflexes, manifested as changes in forces generated between the teeth. It is likely that this technique may produce data which arguably will be more relevant to any possible roles played by reflexes in conditions such as TMD. This is based on the ground that if the protective role is mechanical, then it is the mechanical aspects of the reflex which should be studied.
3.3 Materials and Methods

3.3.1 General

Experiments were performed in the Oral Neurophysiology Research Laboratory, University of Dundee Dental School. The protocol was granted ethical approval by the institutional ethics committee (University Research Ethics Committee, Ref: 8070) and was in accordance with the guidelines of the Declaration of Helsinki of the World Medical Association. There were 15 volunteer subjects (9 males and 6 females; age range 20-40 years; mean 27.1), all of whom gave written, informed consent. All the participants were dentate and reported no symptoms of TMD.

The experiments involved the recording of bilateral masseter EMG and bite force while applying electrical stimulus to the upper lip. The participants were biting on a force transducer.

3.3.2 Recording techniques

In early experiments, EMG activity was recorded using 2 pairs of silver / silver chloride surface electrodes attached to the skin over the masseter muscles. These were held in place using adhesive discs, with an inter-electrode distance of 20 mm. In later experiments, wet-gel, disposable electrodes were used (Ambu® Neuroline 720). The EMG signal was initially processed by amplification (× 10000 - 20000) and filtration (20Hz - 1 kHz) using isolated amplifiers and filters (NL820, NL824 and NL135,
Neurolog, Digitimer, Welwyn Garden City, UK). The signals were then continuously analogue-to-digital converted (2000 digital points per second), using an intelligent interface (CED 1401, Cambridge Electronic Design, Cambridge, UK) connected to a computer running the program Signal (version 2.16; Cambridge Electronic Design, Cambridge, UK). The general recording and stimulus set-up is shown in Figure 2-3.

Bite force was recorded between the incisor and canine teeth in each jaw using a unidirectional force transducer. The force transducer consisted of two metal beams with two strain gauges attached to each side of one beam. The overall thickness of the transducer was 7 mm (Figure 2-1). The force transducer signals were amplified and filtered (DC-50Hz) using a bridge amplifier system (NL107 and NL106, Neurolog, Digitimer, Welwyn Garden City, UK). As with the EMG recordings, the force recordings were continuously analogue-to-digital converted (2000 digital points per second), using the intelligent interface connected to a computer running the Signal program. The transducer was calibrated before each experiment using a series of weights and was found to be linear over a wide range of forces (0 – 196 Newton). The transducer was also checked for its response time and it was found that this was so rapid that the difference between the start of a controlled externally-applied force and the appearance of this force in the recording was less than 0.5 ms, i.e. the time between successive samples in the digitally converted record (Figure 2-2). In order to standardise the position of biting throughout each experiment, self-curing acrylic resin 'indexes' were made on the metal beams of the force transducer for each participant; this also made the transducer more comfortable to use.
3.3.3 Stimulation techniques

In order to evoke reflexes, electrical stimuli were delivered via bipolar skin surface silver / silver chloride disc. These were positioned unilaterally just above the vermilion border of the upper lip and lateral to the philtrum. The stimuli consisted of 1 ms constant-current, rectangular pulses emitted from an isolated stimulator (DS7, Digitimer Ltd.). They were delivered in blocks of ten with a 4 s interval between individual stimulus. The timing was controlled automatically via the interface using the Signal program.

3.3.4 Experimental protocol

At the start of the experiments, a nociceptive threshold for the electrical stimuli was determined. This was defined as the minimum stimulus required to elicit a sharp sensation. In order to evoke the reflex (see below), a range of multiples of the nociceptive threshold was employed, namely 0.7, 1, 1.4, 1.96, 2.74, 3.84, 5.38 & 7.53. This algorithmic scale was found to optimize the building of stimulus/response relationship without the need to apply too many intensities.

The stimuli were applied to the upper lip while the participants produced a controlled level of bite force (target 50N). To this end, the subjects were provided with visual feedback of their force recordings (Figure 2-3). For each stimulation intensity, either 10 (in 6 experiments) or 20 (in 9 experiments) stimuli were delivered (in blocks of 10 as described above).
### 3.3.5 Data processing

The EMG signals were processed by full-wave rectification, averaging and smoothing (Figure 2-4) whereas the bite force recordings were simply averaged (Figure 2-5). The smoothing routine for the averaged EMGs involved the application of a digital filter (low pass 70 Hz at -3 dB). After the records had been processed in this way, the average noise level of the circuit was subtracted; this noise level was estimated by making recordings of the EMG with the jaws slightly open (i.e. with no detectable activity in the jaw closing masseter muscles). The magnitudes of the EMG recordings were normalised as percentages with respect to the mean level of activity in the 200 ms immediately prior to the application of the stimulus (Figure 2-4). A custom-made spreadsheet template (Microsoft Excel) calculated the 5th and 95th percentiles for the same pre-stimulus 200ms period, and these levels were extrapolated into the 200 ms immediately following the stimulus (Figure 2-6). Decreases or increases of activity in the post-stimulus period were considered to be significant inhibitions or excitations respectively if their durations outside the appropriate percentile exceeded 4.7 ms (Van Der Glas et al., 1995); this minimised the risk of labelling chance fluctuations as reflexes. The spreadsheet template was then used to quantify the significant responses. Five parameters were quantified for each wave with respect to the mean pre-stimulus level (see Gardner et al., 2008; Maillou et al., 2010): (1) latencies from the time of stimulation to the onset of the wave (ms); (2) durations (ms); (3) latencies from time of stimulation to peak effect (the maximum reduction for inhibitory waves or maximum increase for excitatory waves) (ms); (4) magnitudes of peak effects –
expressed as percentages of the pre-stimulus baseline activity; (5) integrals with respect to the 100% baseline (%.ms).

Similar analyses were performed on the recordings of bite force except that these recordings were simply averaged and quantified (Figure 2-5) using the spreadsheet (as DC recordings, they did not require to be rectified and they were not smoothed). Because of the slower nature of fluctuations seen in the force records, an extra criterion had to be added when quantifying the responses, namely that an inhibitory reflex was deemed to have started only if the next 5ms of digital values in the force recording were all less than the one before.

3.3.6 Statistical analyses

Summary data are expressed as means ± S.D. The data were analysed using Repeated Measures Analysis of Variance (RM ANOVA) followed, where appropriate, by Bonferroni-corrected paired t-tests. Independent t-test was used to compare the means between genders. In addition, Spearman rank-order correlation was carried out to measure the strength and direction of the association between different parameters’ variables in the EMGs and bite force. P values of less than 0.05 were regarded as significant.
3.4 Results

3.4.1 General

All 15 subjects showed both an inhibition of EMG activity and a decrease in bite force in the period following the application of stimuli of adequate intensity to the lip. In most cases, this was followed by a period during which both the EMG activity and the bite force increased. Examples of such responses from an individual subject are shown in Figure 3-1. Analysis of the quantitative data for these responses revealed no significant differences between the sexes. Accordingly in the detailed descriptions below, the data for males and females have been pooled.
Figure 3-1 Different responses to different intensities of the nociceptive threshold in one subject.
3.4.2 Electromyographic responses

In all but one subject, the EMG responses were produced only by stimuli at or above (sometimes well above) the nociceptive threshold (Figure 3-1 and Figure 3-2). As already described, the responses always consisted of a period of decreased EMG activity and in 93% of the cases, this was followed by a period of increased activity (Figure 2-6). Although the stimuli were applied unilaterally, there were no significant differences in the properties of the responses seen on the two sides (ipsi- and contra-lateral to the stimuli) – either in terms of their timings or of their magnitudes, P > 0.05 paired sample t-test (Table 3-1).
Figure 3-2 Bar Chart is showing the cumulative percentage of subjects showing responses at different stimulus intensities.
The timings (latencies and durations) of the inhibitions and excitations as seen in the EMG recordings are shown in Figure 2-6. The latency (from the stimulus to onset of inhibition) and duration (from the onset of inhibition wave to end point) of the reflex were calculated objectively using the spreadsheet described in Chapter 2. When the minimum latency obtained for each subject for the inhibition was considered, the mean values were 35.37 ± 4.09 and 36.27 ± 4.92 ms for the ipsilateral and contralateral masseters respectively. The equivalent values for the maximum durations were 55.9 ± 10.35 and 54.77 ± 10.35 ms respectively.

Both the depths and the magnitudes (measured as integrals) of the inhibitory reflexes increased with stimulus intensity up (Figure 3-3). Again the values were similar for the two sides with maximum depths 84.87 ± 14.91 and 90.13 ± 8.18 % in the ipsilateral and contralateral EMGs respectively. The equivalent values for the magnitudes (integrals) were 3264.73 ± 1219.15 and 3473.59 ± 1056.86 %ms.
The excitatory EMG responses were much more variable in magnitude than the inhibitions and indeed were absent in 7\% of cases. The mean latencies, durations, and magnitudes (integrals) of the largest of these responses in the 14 subjects who showed them (in one or both of the masseter muscles), were 86.50 ± 8.96 ms, 61.65 ± 18.33 ms and 2991.13 ± 1461.19 % ms respectively for the ipsilateral masseter and 85.54 ± 9.51 ms, 62.75 ± 14.73 ms and 2445.54 ± 922.53 %ms respectively for the contralateral masseter.
3.4.3 Responses seen in force recordings

Clear changes were seen in the force recordings in every case in which there was a change in the EMG. However, the timings of the responses seen in the force records were slower and longer-lasting and the depths of the waves representing muscle relaxation, were less, than those seen in the EMGs.

Because of the slow nature of the evoked changes in the bite force, it was not always easy to determine timings such as latency with the same precision as for the EMGs. It was for this reason that 20 rather than 10 sweeps were averaged for each stimulation intensity in later experiments; however, that did not significantly improve the precision. An independent-sample t-test was conducted to compare the different reflex parameters between the two groups, namely 10 and 20 sweeps groups, and no significant difference was found between the two groups (P > 0.05, two-tailed). Nevertheless, the timings when the minimum latency obtained for each subject for the relaxation in bite force was considered, the mean value was 49.50 ± 6.75 ms. The mean maximum duration of the relaxation in the bite force was 115.50 ± 35.47 ms.

Again considering the maximum values obtained in each subject, the mean depth and integrals for the relaxation responses were 16.06 ± 11.01 % and 910.61 ± 592.71 %.ms respectively.

As with the EMG recordings where the inhibitions were followed by apparent excitations, the relaxations in bite force were usually followed by increases in bite force. This was observed in 13 out of 15 subjects. The long-lasting nature of many of these
responses made it difficult to determine their duration or magnitude with precision. It was therefore, decided to measure only their latencies and peak force levels. Again based on the maximum responses obtained in each person, these were 140.27 ± 17.43 ms and 109.9 ± 6.15 % respectively (Table 3-2).

3.4.4 Comparison of responses in EMGs and force recordings

As described above, the EMG and force records were similar in the general pattern but not in the timings or magnitudes of the evoked responses. As one would expect, the mechanical events (i.e. bite force) were of longer duration than the corresponding electrical (i.e. EMG) events (Figure 2-6).

In order to allow a direct comparison between EMG and bite force responses evoked by a given stimulus, the largest response was identified for each subject in terms of the integral of the response in either EMG recordings. Data for that stimulus intensity was then collated in that individual for all the parameters which we measured – namely the latency, duration, depth and integrals of inhibitory waves and the latency and peak level of the excitatory responses (Table 3-2).

Analyses of these data (ANOVAs followed by post hoc Bonferroni-corrected paired t tests) revealed significant differences between each EMG value and the corresponding bite force one for all parameters (P always less than 0.0005). However, there were never significant differences between the values from the ipsi- and contra-lateral masseter EMGs.
Most notably the maximum durations of the inhibitory waves in the EMGs were of much shorter duration but deeper than the equivalent reductions in bite force. Interestingly the onset of the inhibitory responses invariably occurred earlier in the EMGs than in the bite force recordings.
<table>
<thead>
<tr>
<th></th>
<th>EMG (ipsilateral)</th>
<th>EMG (contralateral)</th>
<th>Bite Force</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inhibitory</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Minimum latency (ms)</td>
<td>35.37 ± 4.09</td>
<td>36.27 ± 4.92</td>
</tr>
<tr>
<td></td>
<td>Maximum duration (ms)</td>
<td>55.90 ± 10.35</td>
<td>54.50 ± 10.35</td>
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<tr>
<td></td>
<td>Maximum depth (% to which parameter fell)</td>
<td>15.13 ± 14.91</td>
<td>9.87 ± 8.18</td>
</tr>
<tr>
<td></td>
<td>Maximum integral (%.ms)</td>
<td>3264.73 ± 1219.15</td>
<td>3473.59 ± 1056.86</td>
</tr>
<tr>
<td><strong>Excitatory</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Minimum latency (ms)</td>
<td>86.50 ± 8.96</td>
<td>85.54 ± 9.51</td>
</tr>
<tr>
<td></td>
<td>Maximum height (% to which parameter rose)</td>
<td>193.24 ± 60.34</td>
<td>181.02 ± 47.13</td>
</tr>
</tbody>
</table>

Table 3-1 Table summarising maximum responses from all subjects. Data are presented as means ± S.D. N=15 (unless otherwise stated). Note that data for durations and integrals are not included for the excitations as in some subjects, the excitations on the force records outlasted the period of the recording.
<table>
<thead>
<tr>
<th></th>
<th>EMG (ipsilateral)</th>
<th>EMG (contralateral)</th>
<th>Bite Force</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inhibitory responses</strong></td>
<td>Latency (ms)</td>
<td>38.17 ± 3.58</td>
<td>38.97 ± 4.49</td>
</tr>
<tr>
<td></td>
<td>Duration (ms)</td>
<td>53.33 ± 10.24</td>
<td>52.33 ± 9.40</td>
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<td>Depth (% to which parameter fell)</td>
<td>15.78 ± 14.40</td>
<td>10.39 ± 7.92</td>
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<tr>
<td></td>
<td>Integral (%.ms)</td>
<td>3216.28 ± 1246.99</td>
<td>3463.65 ± 1046.24</td>
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<tr>
<td><strong>Excitatory responses</strong></td>
<td>Latency (ms)</td>
<td>91.75 ± 10.94</td>
<td>90.04 ± 10.30</td>
</tr>
<tr>
<td></td>
<td>Height (% to which parameter rose)</td>
<td>193.24 ± 60.34</td>
<td>181.02 ± 47.13</td>
</tr>
</tbody>
</table>

Table 3-2 Table summarising data from the largest responses in each subject (as judged from the integrals of the EMG inhibitions). Data are presented as means ± S.D. N=15 (unless otherwise stated). Note that data for durations and integrals are not included for the excitations as in some subjects, the excitations on the force records outlasted the period of the recording. ANOVA followed by Bonferroni-corrected paired t-tests showed no significant differences between any of the parameters of the two EMGs. However, significant differences (P < 0.0005) were obtained in every case for comparisons between each EMG parameter and the corresponding one for bite force.
3.4.5 Correlation between EMG and force measurements

A Spearman's rank-order correlation was run to assess the relationship between ipsi- and contra-lateral EMG and bite force for all parameters. Preliminary analysis showed the relationship to be monotonic, as assessed by visual inspection of a scatterplot.

When analysing the responses in all subject there was a significant strong correlation between ipsi- and contra-lateral EMG latencies $r_s(49) = 0.527$, $P < 0.0005$. Similarly, for all other parameters (i.e. duration, peak and size) there was a significant strong positive correlation between ipsi- and contra-lateral EMG: $r_s(49) > 0.69$, $P < 0.0005$.

For the correlation analyses between EMG and bite force, there was a significant strong correlation between the size of the ipsi- and contra-lateral EMG and the size of the bite force relaxation (Figure 3-4), $r_s(52) = 0.587$, $P < 0.00005$ and $r_s(5) = 0.696$, $P < 0.00005$ respectively. Similar significant strong correlations were obtained for the other parameters – except for the latencies and durations of ipsilateral EMG and bite force where there was a significant moderate relationship (for more details see Table 3-3).
Figure 3-4 Scatter plot for the EMG and bite force integrals (ms%) correlation.

The blue dots represent the ipsilateral EMG × bite force, while the red dots represent the contralateral EMG × bite force.
<table>
<thead>
<tr>
<th></th>
<th>Bite force</th>
<th>Contralateral EMG</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Latency</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ipsilateral EMG</td>
<td>0.470</td>
<td>0.527</td>
</tr>
<tr>
<td>Contralateral EMG</td>
<td>0.579</td>
<td></td>
</tr>
<tr>
<td><strong>Duration</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ipsilateral EMG</td>
<td>0.382</td>
<td>0.700</td>
</tr>
<tr>
<td>Contralateral EMG</td>
<td>0.681</td>
<td></td>
</tr>
<tr>
<td><strong>Peak</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ipsilateral EMG</td>
<td>0.527</td>
<td>0.774</td>
</tr>
<tr>
<td>Contralateral EMG</td>
<td>0.589</td>
<td></td>
</tr>
<tr>
<td><strong>Size</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ipsilateral EMG</td>
<td>0.587</td>
<td>0.875</td>
</tr>
<tr>
<td>Contralateral EMG</td>
<td>0.696</td>
<td></td>
</tr>
</tbody>
</table>

**Table 3-3** Spearman correlation co-efficient for main study variables when analysing responses in all subjects. All results are statistically significant at \( P < 0.005 \) level.
3.5 Discussion

The aim of these experiments was to define the mechanical properties of the inhibitory jaw reflex evoked by peri-oral stimuli and to compare these mechanical properties to the well-established EMG pattern for this inhibitory reflex.

The results are consistent with those of many previous studies in demonstrating that electrical stimulation of cutaneous primary afferents in the hairy skin of the upper lip, can produce a monophasic inhibitory reflex in the masseter muscles while they are active (Yu et al., 1973; Cadden and Newton, 1988). This monophasic peri-orally evoked reflex is easier to quantify and analyse compared to the more complicated biphasic intra-orally evoked reflexes. The electrical stimuli used in these experiments were applied at a frequency of one stimulus every 4 seconds to evoke the reflex. Although, this stimulation frequency might produce slight habituation of the reflex according to Desmedt and Godaux (1976), increasing the peri-stimulus time could have led to a muscle fatigue which in return could have affected the results.

In addition to the well-established inhibitory reflex which can be seen in the EMGs of jaw-closing muscles following nociceptive stimulation of the upper lip, it was demonstrated that it is accompanied by a clear but proportionately smaller drop in forces between the teeth. The onset of the mechanical response was delayed by comparison with the EMG response and lasted much longer. These findings are compatible with previous studies of other inhibitory jaw reflexes (e.g. Yemm, 1972a; Yang and Turker, 1999) although there are no previous investigations which have examined the
mechanical manifestation of the lip-evoked inhibitory reflex (for more details see Chapter 1).

3.5.1 Technical considerations

All subjects were provided with visual feedback from the oscilloscope on which the biting force was displayed as a line. This enabled the baseline activity to be controlled throughout the experiments. In the preliminary phase of the experiment (pilot studies not reported here), 20% of the maximum voluntary biting force between the anterior teeth was used as a baseline. However, the metal force transducer failed to record the maximum biting force in a number of subjects. This was mainly with subjects having a very high biting force that caused the beams of the metal transducer to bend and touch each other. Because of this mechanical limitation, 50 Newton in the bite force was chosen as a baseline for all subjects instead of 20% of the maximum voluntary bite force in each subject. The maximum voluntary bite force values, that have been recorded between the anterior teeth, ranged between 120 N and 350 N (Helkimo et al., 1977; Tortopidis et al., 1998). Therefore, the baseline (50 N) used in the experiments included in this thesis would range from 14% to 42% of the reported maximum voluntary bite force. This possible variation in the clenching level/baseline should not be a problem as long as all comparisons in the results are done for normalised data. It has been found that clenching level does not affect the percentage of total EMG activity inhibited by a given stimulus (Ballantyne et al., 2005). Therefore, when the EMG voltage (and
probably bite force levels) is normalised as percentages of the pre-stimulus level of activity, the reflex magnitude (expressed in percent milliseconds) would remain constant with a different clenching level.

Avoiding the maximum voluntary bite force recording at the start of each experiment could have an advantage of minimising the effect of fatigue. Although, it has been shown previously that segmental conditioning stimuli in the form of a static jaw muscle exercise, has a small but not quite significant effect on the size of the inhibitory jaw reflex elected by an electrical stimulation of the upper lip (Maillou and Cadden, 2008).

3.5.2 Nature of the relaxation

The relatively small drop in bite force between the teeth, as a result of the electrical stimulation on the upper lip, is a clear mechanical manifestation of the electromyographic reflex response in the jaw muscles. It is less likely that the jaw depressor muscles could have contributed to the drop in bite forces via an opening reflex. The existence of an active jaw-opening reflex in human beings is a controversial subject. Yemm (1972a) and Matthews (1976a) reported no digastric or suprahyoid muscle activation accompanying the jaw inhibitory reflex evoked by noxious stimulus to the human oral mucosa. On the contrary, it has been found that trains of high intensity stimuli, usually described as unpleasant, were required to evoke the active opening reflex in man (Cadden et al., 1997). However, this reflex shows a great deal of habituation rate (Desmedt and Godaux, 1976; Cadden et al., 1997). So even if there was
a jaw opening muscle reflex it would have occurred only on the first one or two sweeps each time. The later has been dismissed by examining the bite force recordings. No significant difference in bite force reduction between the first and later sweeps (P > 0.05, paired t-test). The other potential jaw depressor is the inferior head of the lateral pterygoid muscle. Largely because of relative problematic access to the lateral pterygoid comparing with the digastric or other suprahypoid muscles, most of the attention in investigating the active jaw opening reflex has focused on the later. Widmer (1987) showed that the jaw-opening reflex excitation and inhibition in the inferior head of the lateral pterygoid muscle were only detected during activation (i.e. protrusion or opening against resistance) and not when the muscles were relaxed or minimally active (i.e. clenching).

For these reasons, it is unlikely that the digastric and other suprahypoid muscles and/or the lateral pterygoid muscle have contributed to the reduction in bite force and it can only be explained by the silent period in the inhibitory reflex in the jaw elevator muscles.

3.5.3 EMG vs. force

These results show as one would expect that the mechanical parameters of these jaw reflexes are more prolonged than the electromyographic ones. In all 15 subjects, stimulation of the lip above a given level produced both a reduction in the biting force
and a suppression of the masseter EMGs when the maximum stimuli were applied (2.16-5.7mA, 1ms).

The contraction time (usually determined from baseline to the peak of force development) and the subsequent relaxation time depend on the contractile properties of skeletal muscles. Biologically the force-time characteristics are believed to be related to the release and reuptake of Ca^{2+} by the sarcoplasmic reticulum (for more details see below). We were able to show that the onset of reflex relaxation in bite force following stimulation of the human lip lags 13 milliseconds, on average, behind the corresponding reductions in electromyographic activity in one of the primary muscles responsible for generating the force.

The mean amplitude of the reflex was substantially less in the force records than in the EMGs regardless of whether the amplitude was measured as peak value or integral. This can be interpreted by the relativity short duration of the inhibitory reflex, 54.45 ms on average, and by the slow nature of the mechanical manifestation of the electromyographic activity. Therefore, the bite force starts to rise again before completing the relaxation phase.

As detailed in Table 3-3, there was a strong correlation between the integrals (%ms) of the inhibitory reflex of the ipsi- and contra-lateral EMG and the integral of the drop in bite force when analysing all the responses. There was a stronger correlation in all parameters between bite force and contralateral EMG comparing to the same correlations with the ipsilateral EMG. This could be due to the effect of an artifact from the ipsi-lateral electrical stimulation. However, relating the amplitude of EMG to the
amplitude of force could be problematic as many factors influence the force which an active motor unit produces (e.g. muscle length, velocity and number of muscles acting as agonists, antagonists or synergists) (Perry and Bekey, 1981; Roberts and Gabaldon, 2008; Disselhorst-Klug et al., 2009). In addition to that, there is a considerable intersubject variation and a possibility for an electrical cross-talk from adjacent muscles which could contribute to the behaviour of the relationship between EMG and force (Basmajian and De Luca, 1985).

3.5.4 The delay in the relaxation onset

The EMG-force temporal relationship is quite complex. The mechanical event (twitch) associated with a motor unit contraction, is known to be significantly slower than corresponding electrical events i.e. the motor unit action potentials (MUAP). The force or tension does not start to develop until the end of the latent period; 2-3 ms following the stimulus. The maximum of the force twitch is not reached until 50 to 100 ms after the peak of the MUAP, and the decline of the muscle force is much slower than that of the electrical activity (for review see: Perry and Bekey, 1981). This delay, which is usually referred to as electromechanical delay (EMD), can be attributed to several components, all of which are linked to the generation of force in the skeletal muscle. These include: (i) the conduction time for MAUP along the T-tubule system in muscle fibre; (ii) the time required for Ca\(^{2+}\) release by the sarcoplasmic reticulum; (iii) Ca\(^{2+}\) binding to Troponin C (TnC), (iv) cross-bridge activation between myosin and thin
filaments, (v) force development in the contractile component (CC) of the muscle fibre; (vi) time required for the series elastic component (SEC) to be stretched by CC (Cavanagh and Komi, 1979). In a single smooth-muscle cell, it has been found that the latent period between electrical stimulation and the onset of contractile activation is even much longer than that seen in skeletal muscles, and which is believed to be as a result of the step(s) linking Ca\(^{2+}\) to the light chain phosphorylation on the myosin neck (Yagi et al., 1988).

In the present study, we showed that the timings of the responses seen in the force records were significantly slower and longer-lasting comparing to those seen in the EMGs. More interestingly, when analysing the largest responses in each subject, we have found that the onset of reflex relaxation in bite force following stimulation of the human lip lags 13 milliseconds, on average, behind the corresponding reductions in electromyographic activity in the masseter muscle (Figure 3-5). This cannot be due to any inertia in the force transducer as we tested the response time of the force transducer in the laboratory by applying a controlled push using a force pusher. There was no more than 0.5 ms delay in the reaction time of the force transducer (Figure 2-2). Such a lag in the jaw reflex relaxation time has previously been reported but not on this scale for this particular reflex (Yemm, 1972a; Brinkworth and Türker, 2005; Yang and Turker, 1999). The delay between the EMG activity and force decrease during relaxation has been reported in other human muscles under different conditions and by using different methods for calculation (Corser, 1974; Viitasalo and Komi, 1981b; Vos et al., 1990; Ferris-Hood et al., 1996; Leung and Xiao, 1997; Blanpied and Oksendahl, 2006; Ce et al., 2013). This delay, which sometimes is referred to as relaxation electromechanical
delay (R-EMD), ranges from 23ms to about 250ms, (for full list of these studies please refer to Table 3-4). This variability in the R-EMD has been explained by the different: (i) methodologies for calculating the end point (half relaxation time vs. return to baseline); (ii) relaxation modality (voluntary vs. reflex); and/or (iii) sites of recording. Leung and Xiao (1997) calculated the R-EMD as the time difference between cessation of EMG activity and the end of relaxation, while Ferris-Hood et al. (1996) chose 10% of the slope of the relaxation torque curve as detection point. In both studies, the R-EMD value was much higher compared to the other studies. In one particular study, Ce et al. (2013) referred to the delay between the cessation of neuromuscular activity and the initial force decay as the relaxation electrochemical delay (Δt EMG-F) which they said is the first component of the R-EMD and found it to be in an average of 23 ms.
Table 3-4 Summary of previous studies on the relaxation delay. MAP: muscle action potentials

<table>
<thead>
<tr>
<th>Authors</th>
<th>n.</th>
<th>Muscle</th>
<th>Protocol</th>
<th>Contraction</th>
<th>Measurement method</th>
<th>Measured delay (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yemm (1972a)</td>
<td>10</td>
<td>Masseter and Temporalis</td>
<td>Inhibitory reflex</td>
<td>Isometric - isotonic</td>
<td>From onset of silent period to start of opening movement</td>
<td>8-12</td>
</tr>
<tr>
<td>Corser (1974)</td>
<td>7</td>
<td>Elbow extensor</td>
<td>Reaction experiment</td>
<td>Isometric</td>
<td>From end of MAP to onset of movement</td>
<td>30-70</td>
</tr>
<tr>
<td>Viitasalo and Komi (1981b)</td>
<td>29</td>
<td>Quadriceps femoris</td>
<td>Reaction experiment</td>
<td>Isometric</td>
<td>From EMG activity cessation to beginning of force decrease</td>
<td>N/A</td>
</tr>
<tr>
<td>Vos et al. (1990)</td>
<td>5</td>
<td>Vastus lateralis</td>
<td>Repeated voluntary contraction</td>
<td>Isometric</td>
<td>Average amplitude points of the EMG of the falling limbs and force signals – using cross-correlation technique</td>
<td>88.8 ± 18.7</td>
</tr>
<tr>
<td>Ferris-Hood et al. (1996)</td>
<td>23</td>
<td>Quadriceps</td>
<td>Reaction experiment</td>
<td>Isometric</td>
<td>From EMG activity cessation to 10% of the slope of the torque relaxation curve</td>
<td>1st test: 249 ± 68 - 276 ± 51 2nd test: 239 ± 46 - 300 ± 59</td>
</tr>
<tr>
<td>Leung and Xiao (1997)</td>
<td>8</td>
<td>Quadriceps</td>
<td>Reaction experiment</td>
<td>Isometric</td>
<td>Time difference between cessation threshold of integrated EMG and end of relaxation (end knee angle)</td>
<td>105 ± 44.48</td>
</tr>
<tr>
<td>Yang and Turker (1999)</td>
<td>9</td>
<td>Masseter</td>
<td>Inhibitory reflex</td>
<td>Isometric</td>
<td>From onset of inhibitory period and onset of bite force reduction</td>
<td>15</td>
</tr>
<tr>
<td>Brinkworth and Türker (2005)</td>
<td>12</td>
<td>Left masseter</td>
<td>Inhibitory reflex (axial</td>
<td>Isometric</td>
<td>From onset of inhibitory period and onset of bite force reduction</td>
<td>8.8</td>
</tr>
<tr>
<td>Blanpied and Oksendahl (2006)</td>
<td>37</td>
<td>Quadriceps femoris</td>
<td>Reaction experiment</td>
<td>Isometric</td>
<td>From end of MAP to initial change in torque</td>
<td>49 (95% CI = 46-52)</td>
</tr>
<tr>
<td>Ce et al. (2013)</td>
<td>17</td>
<td>Gastrocnemius medialis</td>
<td>Supramaximal tetanic</td>
<td>Isometric</td>
<td>From cessation of neuromuscular activity to the initial force decay</td>
<td>23.4 ± 2.7</td>
</tr>
<tr>
<td>Cè et al. (2014)</td>
<td>17</td>
<td>Gastrocnemius medialis</td>
<td>Tetanic stimulation</td>
<td>Isometric</td>
<td>From the last negative peak of EMG to the initial force decay</td>
<td>20.8 ± 1.7</td>
</tr>
</tbody>
</table>
Figure 3-5 normalised EMG and bite force recordings -160 ms following stimulus- in a representative subject showing the relaxation electromechanical delay (R-EMD). The red arrows point to the inhibition peak-points in the EMG and bite force accordingly. The blue arrows point to the end of the inhibition periods in the EMG and bite force accordingly.
Unlike the contraction delay, fewer studies investigated the delay during the relaxation phase. The relaxation of skeletal muscles is known to comprise: (i) the cessation of the neuromuscular activation; (ii) Ca\(^{2+}\) reuptake by the sarcoplasmic reticulum; (iii) the block of the cross-bridge activation by troponin and tropomyosin; (iv) the release of the SEC which has been previously stretched during contraction; and (v) the return of the sarcomeres to their resting length (Schmidt et al., 1985). The time course of the decay of the active state has been of prime interest in the study of muscle relaxation. Previous attempts were made to explain the delay in the onset of the relaxation and it was thought to be attributed to the rate of Ca\(^{2+}\) reuptake by the sarcoplasmic reticulum rather than to myosin ATPase activity (Viitasalo and Komi, 1981b; Ce et al., 2013). However, this interpretation can be related to the explanation for the slow phase of relaxation, but not to the delay in onset. In skinned frog skeletal muscle fibres, it has been found that the rate and duration of the slow phase of relaxation is believed to be determined in part by the rate of Ca\(^{2+}\) dissociation from the thin filaments (Wahr et al., 1998).

It has been well documented that intact skeletal muscle fibres relax, particularly after a tetanic contraction, in two phases: a slow linear phase during which the fibre remains isometric (isometric relaxation) and ending with a so-called shoulder, and then a much faster exponential phase of force decline (Huxley and Simmons, 1973; Edman and Flitney, 1982; Gillis, 1985). The slow phase is believed to be governed by the rate at which the thin filament deactivates as Ca\(^{2+}\) is removed from troponin C (TnC) (Hoskins et al., 1999).
In a study of changes in sarcomere length and tension during relaxation from isometric tetani in isolated muscle fibres of frog, Edman and Flitney (1982) divided the first slow phase into two components: an initial one, immediately following the last stimulus, when isometric force is maintained at its peak tetanic value; and a later component, corresponding with the slow tension decay. This delay in the onset of the relaxation, is thought to be attributed to the level of free Ca\textsuperscript{2+} which exceeds that required to fully saturate the contractile system by a factor of two, when stimulated maximally (Edman and Flitney, 1982). According to these assumptions, the force should stay for a time, after the last stimulus, at the peak tetanic level until the free Ca\textsuperscript{2+} reaches a point under the mechanical saturation level. Therefore, with a large excess of Ca\textsuperscript{2+} available to the myofibrils during tetanic contraction, it seems that the “level” and not the “rate” of free Ca\textsuperscript{2+} reuptake would be of primary importance in determining the onset of relaxation (Brody, 1976). However, it is difficult to make direct connection between the later findings, which are related to isolated muscle fibres under tetanic contraction, and those of living muscle fibres under more normal physiological conditions, i.e. sub-tetanic contraction. In addition to that, there is some evidence to suggest that the rise in free Ca\textsuperscript{2+} as a result of membrane depolarization, in contraction, has a strong negative-feedback effect on continued Ca\textsuperscript{2+} release from sarcoplasmic reticulum and causes degradation of free Ca\textsuperscript{2+} level (Simon et al., 1991). According to Baylor and Hollingworth (2003), this negative-feedback effect probably prevents Ca\textsuperscript{2+} from exceeding the concentration required to saturate the Ca\textsuperscript{2+} regulatory sites on troponin and therefore avoids unnecessary delays in fibre relaxation.
It has been suggested that due to an extrapolation of thin-filament activation by rigor heads of myosin, the cooperative effect of strongly bound actomyosin cross bridges increases Ca\(^{2+}\) binding to the thin filament (i.e. TnC), and thus increase activation (Wahr et al., 1998; Stehle et al., 2009). It has been shown that feedback mechanisms between attached rigor cross-bridges and thin filament activation can sustain thin filament activation by a co-operative mechanism and can also enhance Ca\(^{2+}\) binding to TnC, which then leads to a decreased dissociation rate of Ca\(^{2+}\) from TnC (Greene and Eisenberg, 1980; Swartz and Moss, 1992; Thirlwell et al., 1994; Brandt and Schachat, 1997; Wahr et al., 1998; Cantino and Quintanilla, 2007). Therefore, during relaxation from maximal tetanic force this cross-bridge-induced thin filament activation (i.e. rigor cross-bridges) may delay force relaxation (Gordon et al., 2000; Tesi et al., 2002; Poggesi et al., 2005). However, there is no convincing evidence to suggest that cycling cross-bridges in skeletal muscles produce these results after Ca\(^{2+}\) removal (Gordon et al., 2000). Conversely, there is some evidence to suggest that that cycling cross-bridges may affect the TnC structure in skeletal thin filaments and may also promote additional Tm movement over the actin surface and expose more myosin-binding sites on actin (for review see Gordon et al., 2000), although it is not very clear how significant these effects are during the first phase of relaxation. Therefore, it is difficult to suggest that cycling cross-bridges kinetics, as seen under normal physiological conditions, contribute to the delay in the onset of relaxation after the removal of the free Ca\(^{2+}\) by sarcoplasmic reticulum in skeletal muscles.

Finally, Huxley and Simmons (1970; 1973) showed that during the first phase of relaxation there may be some cross-bridges going through reattachment and not only
detachment, as the end sarcomeres relaxed first and were stretched while the central sarcomeres shortened.

In summary, most of the data we have for the control of relaxation came from studies of isolated intact or skinned fibres when relaxing from an isometric tetanic contraction. This provides a controlled condition for relaxation studies. Taken together, all these observations could provide some evidence on the kinetics during the first phase of relaxation when sarcomeres are under “relatively” isometric condition. The positive feedback mechanism of cycling or strongly attached cross-bridges could prolong the activation state, and therefore allow for cross-bridge reattachments when some cross-bridges are already going through detachment process. There are no scientific data to suggest that there is a substantial delay between the deactivation of the cross-bridge system (i.e. actomyosin interaction) and the disappearance of the mechanical responses (Gillis, 1985). However, the question whether there is a delay between Ca$^{2+}$ re-uptake by sarcoplasmic reticulum, and the return of actomyosin to the dissociated state has not been fully answered.

3.5.5 The post-inhibitory excitation

The EMG excitation which usually (but not always) follows the inhibition is accompanied by a clear increase in force between the teeth. The results show that increases in bite force usually followed the relaxations in bite force. Interestingly, the discrepancy between force and EMG recordings was much greater for the excitatory
than for the inhibitory responses. This could be due to the slow nature of the mechanical event and because after 250 ms following stimulus it would be nearly impossible to distinguish between voluntary and non-voluntary activities. It was therefore decided to measure only their latencies and peak force levels (Table 3-2).

The reason behind this post-inhibitory reflex is not fully understood. Three main theories have been suggested to explain this phenomenon. Yemm (1972a) suggested that the excitation is a monosynaptic stretch reflex evoked by the preceding inhibitory reflex allowing stretching of the spindles in the jaw closers. Another hypothesis suggests that the excitation is a polysynaptic excitatory reflex evoked directly by stimulation of trigeminal primary afferent fibres (van der Glas et al., 1984b). Finally, Miles et al. (1987) proposed that the excitation is a rebound of activity caused by an inherent property of the motorneurone membrane.

The interest of these experiments was more focused on studying the inhibitory effect. Nevertheless, the results presented in this chapter do not favour any of the theories mentioned above, but arguably are compatible with them all.
3.6 Conclusion and Follow-on Studies

This study has shown that following the application of stimuli of adequate intensity to the lip, an inhibition of electromyographic activity is accompanied by a clear but proportionately smaller drop in forces between the teeth. The relatively short EMG inhibitory period and the slow nature of the accompanying mechanical events, limit the size of the relaxation in bite force. Therefore, the next chapter will describe experiments to investigate this further by comparing the effects of double stimuli on the behaviour of the mechanical manifestation as a result of the inhibitory jaw reflex.
4 Chapter 4: Mechanical Properties of Inhibitory Jaw Reflexes Evoked by double Stimuli of the Upper Lip in Man

4.1 Introduction

As discussed in chapter 3, the well-established inhibitory reflex which can be seen in the EMGs of jaw-closing muscles following nociceptive stimulation of the upper lip is accompanied by a clear but proportionately smaller drop in forces between the jaws. The relatively short EMG inhibitory period and the slow nature of the accompanied mechanical events, have limited the size and the duration of the relaxation in bite force. As a result of that, the bite force dropped by only about 16%, while the EMG activity dropped by about 87% (for more details see chapter 3). Therefore, this chapter will describe experiments to investigate whether increasing the duration of inhibitory wave in EMG recorded inhibitory reflex, as a result of applying double stimuli with different intervals, would increase the depth of the correspondent bite force relaxation.

Repeated stimulation has been used to study the habituation features of the jaw inhibitory reflex in human (Desmedt and Godaux, 1976; Hansen et al., 2002) or of the opening jaw reflex in animals (Hannam, 1973; Vassel et al., 1986). In these studies, the stimulus intervals usually ranged from 200 ms to several seconds. In this current study, double stimulation is aimed at increasing the size of the inhibitory wave and therefore the repeated stimulation would be applied at a much faster rate. Although the determination of the response latency when using more than one stimulus could be
problematical as one cannot be certain which of the stimuli responsible for the response, the results of using such trains of stimuli showed the same pattern as with the single stimuli (Cadden and Newton, 1988).
4.2 Aim

The aim of this study was to investigate the mechanical properties of the inhibitory reflexes when elicited by double stimuli (with interval of 200 ms or less) to the upper lip and compare these with the properties evoked by single stimuli. We believe the latter technique may produce data which could help us have better understanding of the relationship between electromyographic and force features during the inhibitory jaw reflex.
4.3 Materials and Methods

4.3.1 General

Experiments were performed in the Oral Neurophysiology Research Laboratory, University of Dundee Dental School. The protocol was granted ethical approval by the institutional ethics committee (University Research Ethics Committee, Ref: 8070) and was in accordance with the guidelines of the Declaration of Helsinki of the World Medical Association. There were 12 volunteer subjects (10 males and 2 females; age range 23-47 years; mean 33.2), all of whom gave written, informed consent. All the participants were dentate and reported no symptoms of TMD.

The experiments involved recording bilateral masseter EMG and bite force while applying electrical stimuli to the upper lip. The participants were biting on a force transducer. The general techniques used for EMG and force recordings in these experiments have been detailed in Chapter 2. The following section is describing the actual procedures and protocol for this study.

4.3.2 Recording techniques

EMG activity was recorded using wet-gel, disposable electrodes (Ambu® Neurolne 720). The EMG signal was initially processed by amplification ($\times 10000$ - $20000$) and filtration (20Hz - 1 kHz) using isolated amplifiers and filters (NL820, NL824 and NL135, Neurolog, Digitimer, Welwyn Garden City, UK). The signals were

Bite force was recorded between the anterior 6 teeth in each jaw (i.e. between all the incisors and canine teeth) using a unidirectional force transducer. The force transducer signals were amplified and filtered (DC-50Hz) using a bridge amplifier system (NL107 and NL106, Neurolog, Digitimer, Welwyn Garden City, UK). As with the EMG recordings, the force recordings were continuously analogue-to-digital converted (2000 digital points per second), using the intelligent interface connected to a computer running the *Signal* program. The transducer was calibrated before each experiment using a series of weights and was found to be linear over a wide range of forces (0 – 196 Newton). In order to standardise the position of biting throughout each experiment, self-curing acrylic resin 'indexes' were made on the metal beams of the force transducer for each participant; this also made the transducer more comfortable to use.

4.3.3 Stimulation techniques

In order to evoke reflexes, electrical stimuli were delivered via bipolar skin surface silver / silver chloride disc. These were positioned unilaterally just above the vermillion border and lateral to the philtrum. The stimuli consisted of 1 ms constant-
current, rectangular pulses emitted from an isolated stimulator (DS7, Digitimer Ltd.). The electrical stimuli were delivered randomly in 8 different states (each twice in a block of 16) with a 4 s interval between each stimulus presentation. State 1 (control) consisted on of a single stimulus. States 2 – 8 consisted of double stimuli with 2, 5, 10, 20, 50, 100 and 200 ms intervals respectively. As there were 5 blocks of 16 stimuli, each state was applied 10 times. To eliminate any time-related effects, the different states were controlled automatically and randomized within each block via the interface using the Signal program. After each block of 16 sweeps, subjects were instructed to take 3 minutes break to avoid any fatigue effect on the jaw-closing muscles.

4.3.4 Experimental protocol

In order to determine the reflex threshold at the start of each experiment, a sensory threshold for the electrical stimuli was determined. Then by using a multiplier of 1.4, seven multiples of the sensory threshold were calculated: 1.40, 1.96, 2.74, 3.84, 5.38, 7.53, and 10.54 times. These multiples were used to determine the reflex threshold for each subject.

Transcutaneous electrical stimuli of the sensory threshold multiples were delivered to the upper lip while the subjects were maintaining 50 Newton bite force. The resulting records were processed to determine the reflex threshold. During the main experimental sequences, transcutaneous electrical stimuli of an intensity $1.25 \times$ reflex
threshold were applied -for the different states- to the upper lip while the participants produced a controlled level of bite force (target 50N).

To prevent saturation of the recording system as a result of the pick-up of the electrical test stimulus, a mute was triggered coincidently with the simulator. The NL820 has this mute facility which could be triggered at a specific time programmed in *Signal*. The mute was programmed to start 0.5 ms before the stimulus and last for 1.5 ms. In the double stimuli states, two mutes corresponding to each stimulus were employed.

4.3.5 Data processing

For each state, sweeps were extracted for averaging by using *Signal*, then the EMGs and bite force recordings were processed by using the same techniques explained in chapter 2. Four parameters were quantified for each wave with respect to the mean pre-stimulus level: (1) latencies from the time of stimulation to the onset of the wave (ms); (2) durations (ms); (3) magnitudes of peak effects – expressed as percentages of the pre-stimulus baseline activity; (4) integrals with respect to the 100% baseline (%.ms).
4.3.6 Statistical analyses

Summary data are expressed as means ± standard deviation [unless otherwise stated]. Because the magnitudes and durations of the control reflexes (state 1) were variable between subjects, the magnitudes (as integrals %ms and peaks %) and durations of the double stimulus effect for each subject were calculated by normalising the integral and peak of each double-stimuli state (states 2-8) to the control responses in state 1. Then to analyse any effects of the double stimuli in states 2-8 records, one-sample t-tests were conducted (with a null hypothesis that the magnitude or duration did not differ from 100%). This was followed by a Bonferroni correction of 7 to allow for the multiple-comparison nature of these analyses. Additionally, repeated-measures ANOVA was applied to investigate any possible differences in latency between the different states. Finally, a Spearman's rank-order correlation was run to assess the relationship between the EMGs and bite force parameters.
4.4 Results

4.4.1 General

All 12 subjects showed both an inhibition of electromyographic activity and a decrease in bite force in the period following the application of a stimulus of an intensity 1.25 \times reflex threshold to the upper lip. In most cases, this was followed by a period during which both the EMG activity and the bite force increased. Examples of such responses from an individual subject are shown in Figure 4-1. Analysis of the quantitative data for these responses revealed no obvious differences between sexes. Accordingly in the detailed descriptions below, the data for males and females have been pooled.

In the control sequence (state 1), the application of the single electrical stimulus resulted in a single long latency inhibitory wave in all subjects with a mean latency of 44.92 ± 9.38, 40.25 ± 8.62 and 59.17 ± 8 ms for the ipsilateral and contralateral masseters and bite force respectively. The mean durations of the same responses were: 31.04 ± 10.82, 34.54 ± 11.98 and 80.83 ± 18.29 ms respectively.

For states 2 – 5 (double stimuli with 2, 5, 10 and 20 ms intervals respectively) a single long latency inhibitory wave was recorded in all subjects. In state 6 (double stimuli with 50 ms interval), a second inhibitory reflex in EMGs following the second stimulus was recorded in 25% of the subjects. For states 7 and 8 (double stimuli with 100 and 200 ms intervals respectively), the second inhibitory reflex in EMGs was reported in 67% of the subjects (see states 7 and 8 in Figure 4-1). In all these cases,
the size and duration of the second inhibitory reflex wave as a result of the second stimuli was much smaller than this of the first inhibitory reflex wave. The diminished second response could be related to the same mechanism seen with the habituation effect as a result of repeated stimulation.

Quantifying the second inhibitory reflex in the normalised bite force records objectively was not possible because the relaxation effect started when the bite force level was higher than the mean pre-stimulus level (i.e. 50 Newton). This meant that the second relaxation period in bite force did not go under the pre-stimulus level in most cases. However, the analysis of this second inhibitory wave is beyond the scope of this chapter and therefore will not be discussed further.

It was apparent in the EMG recordings that the inhibitions were followed by clear excitations in all subjects. The same applies to the relaxations in bite force which were usually followed by increases in bite force. However, in state 6 (double stimuli with 50 ms interval) the excitation wave was interrupted by the inhibitory wave of the second stimulation in most cases (see state 6 in Figure 4-1). Again, the analysis of the post-inhibitory excitation is beyond the scope of this chapter and therefore will not be discussed further.

In the current sequence of experiments, the discrepancy between ipsi- and contra-lateral EMG recordings was much greater than what is reported in Chapter 3 and what would be expected from similar experiments. The results from the ipsi-lateral EMG recordings gave longer latencies and smaller integrals comparing to the contra-lateral EMG recordings. A Wilcoxon signed-rank test determined that there was a
statistically significant median difference between ipsi- and contra-lateral EMG latencies ($z = 3.371, P = 0.001$) and integrals ($z = 3.826, P = 0.0001$). A possible explanation for this discrepancy will be discussed in the discussion section of this chapter.
Right EMG | Left EMG | Bite Force
--- | --- | ---
State 1 | One stimulus | 
State 2 | Double stimuli | 2ms interval | 
State 3 | Double stimuli | 5ms interval | 
State 4 | Double stimuli | 10ms interval | 
State 5 | Double stimuli | 20ms interval | 
State 6 | Double stimuli | 50ms interval | 
State 7 | Double stimuli | 100ms interval | 
State 8 | Double stimuli | 200ms interval |
(B)

State 1
One stimulus

State 2
Double stimuli
2ms interval

State 3
Double stimuli
5ms interval

State 4
Double stimuli
10ms interval

State 5
Double stimuli
20ms interval

State 6
Double stimuli
50ms interval

State 7
Double stimuli
100ms interval

State 8
Double stimuli
200ms interval

Peristimulus Time (ms)
Figure 4-1 (A) set of records showing an example of individual subject results. Each state shows ipsilateral (right) and contralateral (left) EMGs and bite force recording. The EMG peristimulus records are averaged, smoothed and normalised. The bite force records are simply averaged and normalised. The dashed red horizontal lines represent the mean prestimulus level of EMG or bite force activity to which the records were normalised. The dashed black vertical lines represent the first stimulus. The dashed green vertical lines represent the second stimulus. State 1 (control) contains one stimulus. States 2 – 8 each contains double stimuli with 2, 5, 10, 20, 50, 100 and 200 ms intervals respectively. The records in (B) are displaying the same bite force records in (A) but scaled to show the changes in different states more optimally.
4.4.2 Effect of double stimuli on reflex magnitude

For all subjects, double electrical stimuli with seven different intervals (i.e. 2, 5, 10, 20, 50, 100, 200 ms) were applied randomly. An example of an individual set of records for a single experiment is shown in Figure 4-1.

As the pooled data suggest (see Figure 4-2 and Figure 4-3), the magnitude (represented as integral %ms or peak %) of the responses seen in ipsi- and contralateral EMGs and bite force were affected by the double stimuli. Generally, the EMG inhibition and bite force relaxation increased in size and depth up to state 4 (double stimuli, 10 ms intervals) then started to go back towards the single stimulus level when the interval between the two stimuli was 20 ms or more.
Figure 4-2 a bar chart representing the pooled data for all 12 subjects. Each bar represents the mean of the integral (normalised to the mean prestimulus level of activity %ms) of the inhibitory reflexes obtained from ipsilateral EMG (blue), contralateral EMG (red) and bite force (green). The error bars represent the standard deviation. State 1 (control) contains one stimulus. States 2 – 8 contain double stimulus each with 2, 5, 10, 20, 50, 100 and 200 ms intervals respectively. The table summarises the mean reflex integrals ± S.D. (ms%).
Figure 4-3 a bar chart representing the pooled data for all 12 subjects. Each bar represents the mean of the reflex peak (normalised to the mean prestimulus level %) obtained from ipsilateral EMG (blue), contralateral EMG (red) and bite force (green). The error bars represent the standard deviation.

State 1 (control) contains one stimulus. States 2 – 8 contain double stimulus each with 2, 5, 10, 20, 50, 100 and 200 ms intervals respectively. The table summarises the mean reflex peak ± S.D. (%).
When the magnitudes (i.e. integral and/or peak) of the reflex in each double-stimuli state (states 2-8) were normalised to the control responses (state 1) in each subject, we were able to demonstrate that integrals significantly increased (for EMG and bite force) in size when the intervals between the double stimuli were 5 or 10 ms (states 3 and 4 respectively), \( P < 0.05 \) (single sample t-test with Bonferroni correction, test value 100). There was no significance difference between the control (state 1) and the other states, except for contralateral EMG in state 5 (20 ms interval). The biggest increase in size was with 10 ms intervals (state 4), when the integrals increased by 100%, 120% and 167% for ipsi- and contralateral EMGs and bite force respectively (for more details see Figure 4-4).

The peak of the bite force relaxation, as a result of the double stimuli, showed a significant increase in depth by about 100% comparing to the single stimulus state (control) when the intervals between the double stimuli were 5, 10 or 20 ms (states 3, 4 and 5 respectively), \( P < 0.05 \) (single sample t-test with Bonferroni correction, test value 100). Again, the biggest increase in depth was with 10 ms intervals (state 4), when the peak increased by 124% (for more details see Figure 4-5). The peak of the contralateral EMG increased in depth significantly when the intervals between the double stimuli were 10 or 20 ms (states 4 and 5 respectively) \( P < 0.05 \). There were no significant changes in the depth of the ipsilateral EMGs \( P > 0.05 \).

The biggest response was reported in state 4 where the depth of the inhibitory reflex peak of the contra-lateral EMG increased to \( 153.96 \pm 47.06\% \) of the control level \( (P = 0.014) \); while peak of the relaxation in bite force increased to \( 223.63 \pm 70.88\% \) of the
control level (P = 0.0006, single sample t-test with Bonferroni correction, test value 100).

![Bar chart showing mean size of normalized integrals](image)

<table>
<thead>
<tr>
<th>State2</th>
<th>State3</th>
<th>State4</th>
<th>State5</th>
<th>State6</th>
<th>State7</th>
<th>State8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ipsilateral EMG P</td>
<td>1</td>
<td>0.08</td>
<td>0.04</td>
<td>0.59</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Contralateral EMG P</td>
<td>0.55</td>
<td>0.003</td>
<td>0.001</td>
<td>0.02</td>
<td>1</td>
<td>0.26</td>
</tr>
<tr>
<td>Bite Force P</td>
<td>0.119</td>
<td>0.02</td>
<td>0.003</td>
<td>0.06</td>
<td>0.66</td>
<td>0.64</td>
</tr>
</tbody>
</table>

Figure 4-4 A bar chart showing the mean size of normalized integrals (percentage of state 1 response), of the inhibitory jaw reflex. Data are presented as mean ± SD of the integrals of the reflex waves in ipsi- and contra-lateral EMGs and bite force, each of which was expressed as a percentage of the control response (state 1, single stimulus) for each subject (shown as dashed horizontal black line represent 100%). The table under the bar chart is showing the P value for each measurement (single sample t-test with Bonferroni correction, test value 100). The highlighted table cells are the significant values.
Figure 4-5 A bar chart showing the mean depth of normalized peak (percentage of state 1 response), of the inhibitory jaw reflex. Data are presented as mean ± SD of the peak depth of the reflex waves in ipsi- and contra-lateral EMGs and bite force, each of which was expressed as a percentage of the control response (state 1, single stimulus) for each subject (shown as dashed horizontal black line represent 100%). The table under the bar chart is showing the P value for each measurement (single sample t-test with Bonferroni correction, test value 100). The highlighted table cells are the significant values.
4.4.3 Effect of double stimuli on reflex timing

As shown in Figure 4-6, the latency of the relaxation of the bite force was behind the latency of the corresponding EMGs by $13.5 \pm 3.41$ ms, when averaging the delay in all states. This result is similar to the finding in Chapter 3. Analyses of these data (ANOVA followed by post hoc Bonferroni-corrected paired t tests) revealed significant differences in the latencies between each EMG value and the corresponding bite force ($P < 0.05$).

A one-way repeated measures ANOVA was conducted to determine whether there was a statistically significant difference in mean latencies for each recording between the different states. The application of double stimuli with different intervals did not elicit statistically significant changes in latencies in EMGs ($P > 0.05$). However, the latency of the relaxation in bite force decreased from $59.17 \pm 2.31$ ms in state 1 (control) to $51.92 \pm 1.61$ ms in state 4 (10 ms interval), a decrease of 7.25 ms, which was not statistically significant, $P = 0.15$ (post hoc analysis with a Bonferroni adjustment).

When the duration (ms) of the reflex in each double-stimuli state (states 2-8) were normalised to the control responses (state 1) in each subject (Figure 4-7), there was a significant increase in the duration of reflex in the EMG and bite force when the interval between the double stimuli was 10 ms (states 4). The duration of the inhibitory reflex in the ipsi- and contra-lateral EMG increased to $141.73 \pm 40.35\%$, and $144.7 \pm 46.93\%$, of the control level respectively ($P < 0.05$); while the duration of the relaxation in bite force increased to $128.32 \pm 27.23\%$ of the control level ($P <$
There were no significant differences in the duration of the responses in the other states; P > 0.05 (single sample t-tests with Bonferroni correction, test value 100).

Figure 4-6 A bar chart representing the pooled data for all 12 subjects. Each bar represents the mean of the reflex latency in milliseconds obtained from ipsilateral EMG (blue), contralateral EMG (red) and bite force (green). The error bars represent the standard deviations. State 1 (control) contains one stimulus. States 2 – 8 contain double stimuli with 2, 5, 10, 20, 50, 100 and 200 ms intervals respectively. The table summarises the mean reflex latency ± S.D (ms).
Figure 4-7 A bar chart showing the mean normalized duration (percentage of state 1 response), of the inhibitory jaw reflex. Data are presented as mean ± SD of the peak depth of the reflex waves in ipsi- and contra-lateral EMGs and bite force, each of which was expressed as a percentage of the control response (state 1, single stimulus) for each subject (shown as dashed horizontal black line represent 100%). The table under the bar chart is showing the P value for each measurement (single sample t-test with Bonferroni correction, test value 100). The highlighted table cells are the significant values.
4.4.4 Correlation between EMG and force measurements

A Spearman's rank-order correlation was run to assess the relationship between EMG and bite force parameters. Preliminary analysis showed the relationship to be monotonic, as assessed by visual inspection of a scatterplot.

There was a significant positive correlation between the duration of the inhibitory wave seen in the ipsi- and contra-lateral EMGs and the peak in bite force (Figure 4-8), $r_s(94) = 0.605$ and $r_s(94) = 0.398$ respectively ($P < 0.0005$).

Additionally, the delay between the onset of the inhibition in EMG and the onset of relaxation in bite force tended to decrease with larger responses, from $16.58 \pm 8.59\text{ms}$ with one stimulus to $10 \pm 3.06\text{ms}$ at state 4 (10 ms interval). Therefore, the relationship between EMG integrals and bite force latency was assessed (Figure 4-9). There was a negative correlation between the size of the inhibitory wave in the ipsi- and contra-lateral EMG (as integral %ms) and the latency in bite force, $r_s (94) = -0.232$, $P < 0.05$ and $r_s (94) = -0.489$, $P < 0.00005$ respectively.
Figure 4-8 Scatter plot for the EMGs duration (ms) and bite force peak (depth of relaxation from the prestimulus level, %) correlation. The blue dots represent the ipsilateral EMG × bite force, while the red dots represent the contralateral EMG × bite force.
Figure 4-9 Scatter plot for the EMG integrals (\% ms) and bite force latency (ms) correlation. The blue dots represent the ipsilateral EMG \times bite force while the red dots represent the contralateral EMG \times bite force.
4.5 Discussion

The aim of these experiments was to define the behaviour of the bite force relaxation when increasing the duration of inhibitory jaw reflex by applying double stimuli with different intervals.

The results are consistent with those of many previous studies in demonstrating that electrical stimulation of cutaneous primary afferents in the hairy skin, of the upper lip, can produce monophasic inhibitory reflex in the masseter muscles while they are active (Yu et al., 1973; Cadden and Newton, 1988). In addition, the results of this experimental sequence are consistent with the results of Chapter 3 in demonstrating that the jaw inhibitory reflex is accompanied by a clear but proportionately smaller drop in forces between the teeth.

4.5.1 Technical considerations

In the current sequence of experiments, we noticed that the discrepancy between ipsi- and contra-lateral EMG recordings was much greater than what we reported in Chapter 3 and what would be expected from similar experiments. The results from the ipsi-lateral EMG recordings gave longer latencies and smaller integrals comparing to the contra-lateral EMG recordings. We believe this could be due to the effect of an artifact from the ipsi-lateral electrical stimulation. It is less likely that any artifact would have an effect on the contralateral EMG recording because of the slightly greater distance. This artifact may have disturbed the ipsilateral EMG recording.
Despite the application of a mute, which was triggered coincidently with the simulator, most of the subjects showed discrepancies in the ipsi-lateral EMG causing a delay in the latency and/or alteration in the magnitude of the reflex (as integral or depth). From the raw data observation, the artifact was even bigger when the prestimulus level of activity was lower. For this technical limitation, the discussion of the results will focus mainly on the contra-lateral EMG and bite force.

4.5.2 Reflex changes

As the pool data suggests (see Figure 4-2 and Figure 4-3), the magnitude (represented as integral %ms or peak %) of the responses seen in ipsi- and contra-lateral EMGs and bite force were affected by the double stimuli. Generally, the EMGs inhibition and bite force relaxation increased in size and depth up to state 4 (double stimuli, 10 ms intervals) then started to decline when the interval between the two stimuli was 20 ms or more.

When the magnitudes (i.e. integral and/or peak) and durations of the reflex in each double-stimulus state (states 2-8) were normalised to the control responses (state 1) in each subject, we were able to demonstrate that integrals, peaks and durations significantly increased (for EMG and bite force) and provided biggest responses when the intervals between the double stimuli were 10 ms (state 4), P < 0.05. In state 4, the duration of the inhibitory reflex in the contra-lateral EMG increased by 44.7%, comparing to the control (state 1); while the duration of the relaxation in bite force
increased only by 28.33%. However, the depth of the inhibitory reflex peak of the contra-lateral EMG increased by 53.96%; while peak of the relaxation in bite force increased by 123.63%. Correlation assessment revealed a significant positive correlation between the duration of the inhibitory wave seen in the ipsi- and contra-lateral EMGs and the peak in bite force (Figure 4-8). These findings suggest that the prolongation of the EMG inhibitory jaw reflex evoked by double stimulation of the upper lip (with 10 ms intervals) results in a greater increase in the depth of the accompanied relaxation comparing to a relatively smaller increase in the duration of the relaxation. Similar pattern was observed in states 2 and 3 (2 and 5 ms intervals respectively).

The substantial increase in the depth of the bite force relaxation was more than double that of the corresponding EMG. Given how large EMG peaks often are even with single stimuli – possibly near saturation, these EMG results are not surprising. While the increase in the inhibitory EMG duration by 54% could have contributed to the further increase of the depth of the relaxation (allowing the muscle to relax longer), the “rate” of relaxation played an important role as well. As discussed previously in Chapter 3, muscles relax when the sarcoplasmic free Ca^{2+} falls, allowing calcium to dissociate from Troponin C (TnC) and the muscle fibre to become deactivated. Intact skeletal muscle fibres relax in two main phases, a slow linear phase during which the fibre remains isometric and then a much faster exponential phase. This transition from slow to fast phase is believed to begin when one end of the muscle fibre suddenly starts to lengthen and the changes of sarcomeres length is disordered (Hoskins et al., 1999; Gordon et al., 2000; Tesi et al., 2002). Therefore, the depth increase seen in the
relaxation (following prolongation of EMG inhibition) could be, at least in part, as a result of increased number of muscle fibres going into the fast exponential phase of relaxation.

The observed effect of double stimulus on the latency of relaxation in bite force was not significant (P > 0.05, repeated measures ANOVA). However, the delay between the onset of the inhibition in EMG and the onset of relaxation in bite force tended to decrease with larger responses, from 16.58 ± 8.59ms with one stimulus to 10 ± 3.06ms at state 4 (10 ms interval). Correlation assessment revealed that there was a moderate negative correlation between the size of the inhibitory wave in the contra-lateral EMG (as integral %ms) and the latency in bite force (Figure 4-9). This could be related to the rate at which the rigor-like cross bridges go from force generating stage to non-force generating stage. Therefore, the sarcomeres length is disordered at a faster rate and making the first linear phase of the relaxation even shorter (for more details see chapter 3).
4.6 Conclusion and Follow-on Studies

This study has shown that following the application of double stimuli of adequate intensity and with an optimal interval, to the upper lip, a significant increase in the duration of the inhibitory jaw reflex seen in the EMG is accompanied with a significant increase in the depth of the bite force relaxation. The increase in the depth of the relaxation peak as a result of the double stimuli was proportionally greater than the increase of the duration of the relaxation.

The following chapter will describe experiments to investigate this reflex further by investigating the effects of an experimental-induce pain/fatigue on the behaviour of the mechanical manifestation of this jaw reflex.
5 Chapter 5: Investigation of the Possible Effects of Accelerated Chewing on a Mechanically-Recorded Human Jaw Reflex

5.1 Introduction

As discussed in Chapter 1, pain due to temporomandibular disorders (TMD) is a significant clinical problem in dentistry. The aetiology of these conditions remains uncertain, with many hypotheses having been proposed over the years. One includes the variations in jaw reflexes as underlying aetiology and/or a result of the TMD itself since the inhibitory jaw reflex has been found to be suppressed in this group of patients (De Laat et al., 1985).

Experimentally-induced pain techniques are usually used to study pain mechanisms under controlled settings (for recent review see Olesen et al., 2012). Because myogenous pain in jaw muscles is one of the symptoms in TMD patients, experimentally-induced pain techniques have been used in the past to study the effect of pain on aspects of somatosensory and motor function in the orofacial region. These experimental methods, to evoke pain under controlled circumstances, could help find answers to the cause-effect relationships, as any subsequent changes in the jaw motor function - in healthy volunteers - are likely to be as a result of the experimental pain (Lobbezoo et al., 2002). Different segmental conditioning stimuli have been used to induce pain (and/or fatigue) experimentally in the orofacial region in order to study the effect of pain on the inhibitory jaw reflex in human. These methods include: (i) laser-induced skin pain (Truini et al., 2006), (ii) sustained muscle contraction
(Maillou and Cadden, 2008), (iii) accelerated chewing task (Maillou et al., 2010), and (iv) intramuscular infusion of hypertonic saline (Wang et al., 1999). However, the reported results of these studies are not consistent. Some studies had found little or no effect of induced pain on the jaw reflexes (e.g. Bendtsen et al., 1993; Truini et al., 2006; Maillou and Cadden, 2008). This could be contributed to fast-transient character of the induced pain in some techniques and/or to the inadequate levels of the induced jaw pain (Lobbezoo et al., 2006). Invasive techniques such as hypertonic saline infusion into the masseter muscle have been found to produce a significant effect on the inhibitory jaw reflex (Wang et al., 1999; Svensson et al., 1999). Similar findings have been obtained with non-invasive techniques. A previous study in our laboratory demonstrated that non-invasive, experimentally-induced pain and fatigue in jaw muscles could alter the size of the inhibitory jaw reflex significantly (van der Kaaij et al., 2009; Maillou et al., 2010).

With muscle fatigue, the shape of muscle action potentials undergo some changes, including amplitude reduction and duration increase (Gandevia, 1995). This change in the action potentials shape would result in EMG spectrum shifting to lower frequencies (Figure 5 10). Previous investigators have shown that the shift in median power frequency is related to the decreasing conduction velocity rate of the muscle fibres and/or decreasing in motor unit discharge rate (Stulen and De Luca, 1982; Krogh-Lund and Jorgensen, 1992). Median power frequency is the most commonly used EMG frequency parameter to detect localized muscle fatigue as it shows lesser sensitivity to noise compared to other frequency parameters (e.g. mean power frequency and ratio) (Stulen and De Luca, 1981). Therefore, the median power
frequency was chosen to describe the power spectrum in the pre-stimulus EMG activity in all subjects in this study.

The suppression of inhibitory jaw reflexes by fatigue and/or pain constitutes a loss of a negative feedback mechanism which is usually believed to minimise the risk of overloading structures involved in mastication – this may in turn contribute to the symptoms of TMD (Orchardson and Cadden, 1998). However, before making such links, it is important to investigate any potential changes in the mechanical manifestations of the jaw reflexes i.e. the change in force between the jaws, in addition to monitoring these reflexes by means of electromyographic (EMG) recordings as was done in the previous studies (De Laat et al., 1985; Türker et al., 1989; van der Kaaij et al., 2009; e.g. Maillou et al., 2010) as logically it would be important for the inhibition to show clear signs of producing less unloading of the masticatory apparatus, not simply less electrical activity in the muscles.
5.2 Aim

As discussed in chapters 3 and 4, the well-established inhibitory reflex which can be seen in the EMGs of jaw-closing muscles following nociceptive stimulation of the upper lip is accompanied by a clear but proportionately smaller drop in forces between the jaws.

This study was undertaken to investigate whether the mechanical manifestation of the inhibitory jaw reflex evoked by stimulation of the human lip, can be modulated by an accelerated chewing task for three minutes. The conditioning procedure used in this experiment is believed it could produce conditions that mimic symptoms of the myogenous temporomandibular disorder.
5.3 Materials and Methods

5.3.1 General

Experiments were performed in the Clinical Oral Neurophysiology Research Laboratory, University of Dundee Dental School. The protocol was granted ethical approval by the institutional ethics committee (University Research Ethics Committee, Ref: 8070) and was in accordance with the guidelines of the Declaration of Helsinki of the World Medical Association (WMA, 2008). There were 23 volunteer subjects (15 males and 8 females; age range 23-47 years; mean 31), all of whom gave written, informed consent. All the participants were dentate and reported no present or previous symptoms of TMD.

The experiments involved recording bilateral masseter EMG and bite force while applying electrical stimuli to the upper lip. The recordings were made before, immediately after, and 5 and 10 min following the conditioning procedure. The participants were biting on a force transducer. The general techniques used for EMG and force recordings in these experiments have been detailed in Chapter 2. The following section is describing the actual procedures and protocol for this study.

5.3.2 Recording techniques

EMG activity was recorded using wet-gel, disposable electrodes (Ambu® Neurolin 720). The EMG signal was initially processed by amplification (× 10000 - 20000) and
filtration (20Hz - 1 kHz) using isolated amplifiers and filters (NL820, NL824 and NL135, Neurolog, Digitimer, Welwyn Garden City, UK). The signals were continuously analogue-to-digital converted (2000 digital points per second), using an intelligent interface (CED 1401, Cambridge Electronic Design, Cambridge, UK) connected to a computer running the program Signal (version 2.16; Cambridge Electronic Design, Cambridge, UK).

Bite force was recorded between the anterior 6 teeth in each jaw (i.e. between all the incisors and canine teeth) using a unidirectional force transducer. The force transducer signals were amplified and filtered (DC-50Hz) using a bridge amplifier system (NL107 and NL106, Neurolog, Digitimer, Welwyn Garden City, UK). As with the EMG recordings, the force recordings were continuously analogue-to-digital converted (2000 digital points per second) using the intelligent interface connected to a computer running the Signal program. The transducer was calibrated before each experiment using a series of weights and was found to be linear over a wide range of forces (0 – 196 Newtons). In order to standardise the position of biting throughout each experiment, self-curing acrylic resin indices were made on the metal beams of the force transducer for each participant; this also made the transducer more comfortable to use.
5.3.3 Stimulation techniques

In order to evoke reflexes, electrical stimuli were delivered via bipolar skin surface silver / silver chloride disc electrodes. These were positioned unilaterally just above the vermillion border and lateral to the philtrum. The stimuli consisted of 1 ms constant-current, rectangular pulses emitted from an isolated stimulator (DS7, Digitimer Ltd.). The electrical stimuli were delivered in blocks of 10 sweeps with a 4 s interval between each stimulus.

To prevent saturation of the recording system as a result of the electrical test stimulus pick-up, a mute within the amplifier was triggered coincidently with the simulator. The NL820 has this mute facility which can be triggered at a specific time programmed in Signal software. The mute was programmed to start 0.5 ms before the stimulus and last for 1.5 ms.

5.3.4 Experimental protocol

In the preparation for the experiments, the habitual chewing rate was measured and documented by asking the participants to chew a 1g bolus of flavourless gum at a rate similar to what they would do while eating. This was followed by recording the mean noise level of the EMG recording circuits.

At the start of each experiment, the sensory perception threshold for the electrical stimulation of the upper lip was determined. To determine the reflex threshold for
each subject, seven multiples of the sensory threshold were calculated: 1.40, 1.96, 2.74, 3.84, 5.38, 7.53 and 10.54 times (i.e. a multiplier of 1.4). These multiples were delivered to the upper lip while the subjects were maintaining a 50 N bite force. The resulting records were processed to determine the reflex threshold. During the main experimental sequences, transcutaneous electrical stimuli of an intensity $1.25 \times$ reflex threshold were applied to the upper lip while the participants produced a controlled level of bite force (again target 50 N). The stimuli were delivered in blocks of 10 at 4 seconds intervals in all the experiment sequences. The first sequence (the control) was carried out before the conditioning procedure.

The conditioning procedure consisted of an accelerated chewing task on 1 g of flavourless gum for a period of 3 min. A metronome was set at a rate that was $1.5 \times$ the previously determined habitual chewing rate. The subjects were asked to chew in synchrony with the pre-set metronome (Maillou et al., 2010). The subjects were asked to state whether they experienced muscle pain and/or muscle fatigue as a result of this procedure.

Immediately following the conditioning procedure, another set of recordings was carried out, followed by two further sets after 5 and 10 min of rest periods.

5.3.5 Data processing

For each experimental sequence, the EMGs and bite force recordings were processed using the same techniques explained in chapter 2. Five parameters were quantified for
each wave with respect to the mean pre-stimulus level: (1) latencies from the time of stimulation to the onset of the wave (ms); (2) durations (ms); (3) magnitudes of peak effects – expressed as percentages of the pre-stimulus baseline activity; (4) integrals with respect to the 100% baseline (%ms) and (5) Relaxation electromechanical delay (R-EMD) in ms.

The median power frequency was chosen to describe the power spectrum in the pre-stimulus EMG activity in all subjects. For the 300 ms pre-stimulus EMG in all experimental sequences, 512-point Fast Fourier Transformation (FFT) routine was carried out using Signal software to transfer the data from the time domain to the frequency domain. Consequently, the median power frequency was calculated using formulae on Excel spreadsheets.

5.3.6 Statistical analyses

Summary data are expressed as means ± standard deviation [unless otherwise stated]. Because the magnitudes and durations of the control reflexes (before the conditioning procedure) were variable between subjects, the magnitudes (as integrals in %ms and peaks in %) and durations (in ms) following the conditioning sequences for each subject were calculated by normalising the integral, peak and duration of each postconditioning sequence to the control responses in the preconditioning sequence. Then to analyse any effects of the conditioning procedure, one-sample t-test was conducted with a null hypothesis that the magnitude or duration did not differ from
100%. This was followed by a Bonferroni correction of 3 to allow for the multiple-comparison nature of these analyses. Additionally, repeated-measures ANOVA was applied to investigate any possible differences in latency and median power frequency between the different sequences. Finally, Fisher’s exact test was used to check for the possibility of gender-related differences and for investigating any association between the occurrence of pain and/or fatigue and the direction of the reflex size changes.
5.4 Results

5.4.1 General

All 22 subjects showed both an inhibition of electromyographic activity and a decrease in bite force in the period following the application of a stimulus of an intensity 1.25 × reflex threshold to the upper lip. In most cases, this was followed by a period during which both the EMG activity and the bite force increased. Examples of such responses from an individual subject are shown in Figure 5-1.

One of the 23 subjects showed high noise to EMG ratio, and therefore was excluded from the analysis. In the control sequence (pre-conditioning), the application of the an electrical stimulus resulted in a single long latency inhibitory wave in all 22 subjects with mean latencies of 40.93 ± 5.71, 40.50 ± 5.66 and 55.65 ± 6.86 ms for the ipsilateral and contralateral masseter EMGs and bite force respectively. The mean durations of the same responses were: 43.02 ± 10.12, 44.07 ± 12.02 and 95.54 ± 26.55 ms respectively. Analysis of the quantitative data for these responses revealed no significant differences between the males and females (P > 0.05, independent samples t-test). Accordingly in the detailed descriptions below, the data for males and females have been pooled.

Immediately following the conditioning procedure, 17 out of 22 subjects reported pain and/or fatigue. However, only 4 of the 17 subjects reported both pain and fatigue in one or both of the masseter muscles. Because of the small sample size, subjects who reported pain will be grouped with subjects who reported fatigue. There was no
significant difference between the presence or absence of symptoms between males and females following the conditioning procedure (P = 1.00, Fisher’s exact test), see Table 5-1 for the cross-tabulation data.
Figure 5-1 An example of individual subject results. Each sequence shows ipsilateral and contralateral EMGs and the bite force recording. The EMG peristimulus records are averaged, smoothed and normalised. The bite force records are simply averaged and normalised. The dashed red horizontal lines represent the mean prestimulus level of EMG or bite force activity to which the records were normalised. The dashed black vertical lines represent the timing of the electrical stimuli. The records show: (a) the pre-conditioning sequence (control); (b) the post-conditioning sequence; (c) 5 min post-conditioning sequence; and (d) 10 min post-conditioning sequence.
## Table 5-1

Cross-tabulation for the pain and/or fatigue occurrence in males and females following the conditioning procedure. “Yes” is when the subjects reported pain and/or fatigue, while “No” when the subjects didn’t report pain nor fatigue.

<table>
<thead>
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<th>% within gender</th>
<th>% within Pain and/or Fatigue</th>
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<table>
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<table>
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<th>Count</th>
<th>% within gender</th>
<th>% within Pain and/or Fatigue</th>
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</table>
5.4.2 Conditioning procedure effect on reflex magnitude

Immediately following the conditioning procedure, the reflex size (integral) decreased in one or both EMGs (i.e. ipsi- and contra-lateral EMG) in 19 subjects and increased in 4. This decrease did not differ between males and females (P = 0.227, Fisher’s exact test), see Table 5-2 for the cross-tabulation data.

There was a mean decrease in the reflex integral (in the data pooled from all 22 subjects) to 92.62 ± 42.76 % and 89.07 ± 29.7 % of the control level for ipsi- and contra-lateral EMG respectively. This decrease in the reflex size was reported in the bite force relaxation immediately following the conditioning procedure in 17 subjects. A magnified force recording showing the inhibitory and excitatory responses in one subject are shown in Figure 5-2. The mean decrease in bite force relaxation was to 76.04 ± 35.63 % of the control level in the 22 subjects (Figure 5-3).

There was a small decrease in the depth of the reflex peak following the conditioning procedure (Figure 5-4). This decline in peak depth in one or both EMGs (i.e. ipsi- and contra-lateral EMG) was reported in 19 subjects. The mean decrease in the peak depth (in the data pooled from all 22 subjects) was to 95.15 ± 32.94 % and 93.03 ± 25.64 % of the control level for ipsi- and contra-lateral EMG respectively. This decrease in the peak depth was reported in the bite force relaxation immediately following the conditioning procedure in 15 subjects. The mean decrease in bite force peak depth was to 88.13 ± 28.03 % of the control level in the 22 subjects.
<table>
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<th>% within Size Change</th>
</tr>
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<td></td>
<td>Bigger</td>
<td></td>
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<td>22</td>
<td>86.4%</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

Table 5-2 Cross-tabulation for the direction of size changes in males and females following the conditioning procedure. “Smaller” implies the reflex integral following conditioning was smaller (or the same) than the integral preconditioning. “Bigger” implies the reflex integral following conditioning was bigger than the integral preconditioning.
Figure 5-2 Peristimulus superimposed bite force recordings -from the same subject in Figure 5 1 (magnified)- showing reflex reductions in force (*) produced by electrical stimuli at time 0 (vertical dotted line) delivered before, immediately after, and 5 and 10 min following the conditioning procedure (accelerated chewing task). Note the smaller size of the relaxation immediately following the conditioning procedure (the blue record). Note the increase in the reflex 5 and 10 minutes following the conditioning procedure. This was not typical behaviour in the rest of the subjects.
Figure 5-3 A bar chart representing the pooled data for all 22 subjects. Each bar represents the mean of the reflex integral (normalised to the mean pre-stimulus level of activity %ms) obtained from ipsilateral EMG (blue), contralateral EMG (red) and bite force (green). The error bars represent the standard deviation. The table summarises the mean reflex size ± S.D. (%ms).
Ipsilateral EMG  63.41 ± 16.90  59.31 ± 19.09  63.07 ± 16.96  61.25 ± 15.44
Contralateral EMG  65.31 ± 16.67  58.92 ± 17.72  65.16 ± 16.95  63.42 ± 19.33
Bite Force  6.61 ± 4.23  6.02 ± 4.62  6.12 ± 4.48  6.44 ± 4.68

Figure 5-4 A bar chart representing the pooled data for all 22 subjects. Each bar represents the mean of the reflex peak (normalised to the mean pre-stimulus level %) obtained from ipsilateral EMG (blue), contralateral EMG (red) and bite force (green). The error bars represent the standard deviation. The table summarises the mean reflex size ± S.D. (%).
When the integrals of the responses in each post-conditioning sequence were normalised to the control responses (pre-conditioning) in each subject, no significant differences in the size of the reflex in the EMGs were found in any of the three post-conditioning experimental sequences (P > 0.05). However, the integral for the bite force relaxation significantly decreased in size immediately following the conditioning procedure by 23.96 ± 35.63% in average, P = 0.014 (single sample t-test with Bonferroni correction, test value 0). Five minutes after conditioning, the reflex size began to recover. Ten minutes following the conditioning procedure the size was back to almost normal level (Figure 5-5). There was no significance difference between the control (pre-conditioning) and the 5 and 10 min post-conditioning experimental sequences; P > 0.05 (Figure 5-5).

When the peak depth of the responses in each post-conditioning sequence were normalised to the control responses (pre-conditioning) in each subject, no significant differences in the EMGs or bite force were found in any of the three postconditioning experimental sequences, P > 0.05 (single sample t-tests with Bonferroni correction, test value 100). The same applied to the experimental sequences 5 and 10 min following the conditioning procedure.
Figure 5-5 A bar chart showing the mean size of normalized integrals (percentage of the pre-conditioning sequence), of the inhibitory jaw reflex. Data are presented as mean ± SD of the reflex waves integrals in ipsi- and contralateral EMGs and bite force, each of which was expressed as a percentage of the control response for each subject (shown as dashed horizontal black line represent 100%). The asterisk above the post-conditioning bite force bar indicates that the reflex integral differs significantly from the pre-conditioned integral, P = 0.014 (single sample t-test with Bonferroni correction, test value 100).
Figure 5-6 Bar chart showing the mean depth of normalized peak (percentage of the pre-conditioning sequence), of the inhibitory jaw reflex. Data are presented as mean ± SD of the peak depth in ipsi- and contra-lateral EMGs and bite force, each of which was expressed as a percentage of the control response for each subject (shown as dashed horizontal black line represent 100%). No significant differences were found between the experimental sequences; P > 0.05 (single sample t-test with Bonferroni correction, test value 100).
A Fisher's exact test for association was conducted between the direction of size change and the presence or absence of pain and/or fatigue following the conditioning procedure. There was a statistically significant association between the occurrence of pain and/or fatigue and the changes of reflex size following the conditioning procedure, \( P = 0.006 \). In the pain/fatigue group 100% of the subjects showed smaller integrals following the conditioning procedure in one or both of the masseter EMGs, while only 40% of the no pain/fatigue group subjects showed smaller integrals; see Table 5-3 for the cross-tabulation data.

<table>
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<tr>
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<td>100.0%</td>
<td>22.7%</td>
</tr>
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<td>86.4%</td>
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<td>100.0%</td>
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</tr>
</tbody>
</table>

Table 5-3 Cross-tabulation for the direction of size changes following the conditioning procedure in those with or without pain/fatigue. “Smaller” implies the reflex integral following conditioning was smaller (or the same) than the integral preconditioning. “Bigger” implies the reflex integral following conditioning was bigger than the integral preconditioning.
In addition, there were significant differences between the two groups (i.e. those with or without pain/fatigue) in the changes in size of the contralateral EMG reflex and bite force relaxation following conditioning (P=0.034 and P=0.038 respectively; independent-samples t-test) – the no fatigue/pain group showing greater increases in the reflex parameters. Curiously, there was no significant difference in the changes of ipsilateral reflex size between the same two groups, P = 0.34.

Within the pain/fatigue group, the sizes of the reflexes in ipsi- and contra-lateral EMGs following conditioning were significantly smaller; P = 0.05 and P = 0.002 respectively (one-way repeated measures ANOVA with Bonferroni-corrected post hoc tests). Similarly, the size of the relaxation in bite force was significantly smaller following the conditioning procedure, P = 0.002.

When the post-conditioning integrals were normalised to the control responses (pre-conditioning) for each subject within the pain/fatigue group (Figure 5-7), it showed that the integrals significantly decreased by 20.59 %, 22.09 % and 36.69 % in average for the ipsi- and contra-lateral EMGs and bite force respectively, P < 0.05 (single sample t-test with Bonferroni correction, test value 0). Five and ten minutes after conditioning, the reflex size began to recover and was not significantly different from the pre-conditioning size, P > 0.05
Figure 5-7 A bar chart showing the mean size of normalized integrals (percentage of pre-conditioning sequence), of the inhibitory jaw reflex for subjects within pain/fatigue group (n=17). Data are presented as mean ± SD of the integrals of the reflex waves in ipsi- and contra-lateral EMGs and bite force, each of which was expressed as a percentage of the control response (shown as dashed horizontal black line represent 100%). The table under the bar chart is showing the P value for each measurement (single sample t-test with Bonferroni correction, test value 100). The highlighted table cells are the significant values.
5.4.3 Conditioning procedure effect on reflex timing

As shown in Figure 5-8, the mean latency of the inhibitory reflex for all subjects (n = 22) increased immediately following conditioning by 5.61 ± 1.72, 3.5 ± 1.72 and 10.2 ± 1.49 ms [mean ± standard error of mean] for the ipsi- and contra-lateral EMGs and bite force, respectively, compared to the pre-conditioning latencies. A one-way repeated measures ANOVA with Bonferroni-corrected post hoc tests revealed that there were statistically significant differences in latencies between pre- and post-conditioning sequences for the ipsilateral EMG and bite force (P = 0.022 and P = 0.000007 respectively). The latency of the ipsilateral masseter reflex remained significantly different 5 min following the conditioning procedure (P < 0.05), while the bite force relaxation latency remained significantly different throughout all the post-conditioning sequences (P < 0.05). The latency increase in the contralateral masseter reflex was not significant. However, when analysing the latencies from the pain/fatigue group (n = 17), all three recordings showed the significant increases in latencies following the conditioning procedure (P < 0.05).

When analysing the delay between the latencies of the reflex in EMGs and bite force relaxation (R-EMD), there was a significant increase in the delay following the conditioning procedure by 5.47 ± 1.60 ms [mean ± standard error of mean], (P = 0.015, Repeated measures ANOVA with Bonferroni-corrected post hoc tests), (Table 5-4).
Table 5-4 The mean ± SD of the relaxation electromechanical delay (R-EMD) between the average latencies of ipsi- and contra-lateral masseter inhibitor reflexes and bite force relaxation in milliseconds. The asterisk indicates that the R-EMD differs significantly from the pre-conditioned one, P = 0.015 (Repeated measures ANOVA with Bonferroni-corrected post hoc tests)

<table>
<thead>
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<th>Pre-conditioning</th>
<th>Post-conditioning</th>
<th>5 min post-conditioning</th>
<th>10 min post-conditioning</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-EMD (ms)</td>
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<td>20.41 ± 5.73*</td>
<td>17.85 ± 7.17</td>
<td>16.73 ± 6.85</td>
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</table>

When the durations (ms) of the reflexes in each post-conditioning sequence were normalised to the control responses (pre-conditioning) in each subject (Figure 5-9), there was a statistically significant decrease in the duration of the relaxation in bite force following the conditioning procedure - to 83.60 ± 19.65 % of the control level, P = 0.003 (single sample t-test with Bonferroni correction, test value 100). On average, the normalised durations of the reflexes in the ipsi- and contra-lateral EMGs were smaller following the conditioning procedure, but these differences were not significant. However, when analysing the normalised duration of the responses in the pain/fatigue group (n = 17), the duration of the inhibitory reflex in the contralateral masseter EMG decreased significantly following the conditioning procedure to 77.79 ± 19.21% of the control level, P = 0.0006. For the same group, the normalised duration of the bite force relaxation decreased significantly following the conditioning
procedure to $76.79 \pm 11.7\%$ of the control level, $P < 0.00005$ (single sample t-test with Bonferroni correction, test value 100).

Ipsilateral EMG $40.93 \pm 5.71$ $46.55 \pm 7.63$ $45.48 \pm 7.21$ $44.93 \pm 6.38$

Contralateral EMG $40.50 \pm 5.66$ $44.00 \pm 8.78$ $44.77 \pm 8.41$ $44.66 \pm 9.02$

Bite Force $55.66 \pm 6.86$ $65.68 \pm 8.43$ $62.98 \pm 11.24$ $61.52 \pm 9.32$

Figure 5-8 A bar chart representing the pooled data for all 22 subjects. Each bar represents the mean of the reflex latency in milliseconds obtained from ipsilateral EMG (blue), contralateral EMG (red) and bite force (green). The error bars represent the standard deviation. The table summarises the mean reflex latency $\pm$ S.D (ms). The asterisks above the bars indicate that the reflex latency differs significantly from the pre-conditioned latency, * $P < 0.05$, ** $P < 0.00005$ (repeated measures ANOVA with Bonferroni corrected post hoc tests).
Figure 5-9 A bar chart showing the mean normalized duration (percentage of the pre-conditioning sequence), of the inhibitory jaw reflex in all subjects (n = 22). Data are presented as mean ± SD of the duration of the reflex waves in ipsi- and contra-lateral EMGs and bite force, each of which was expressed as a percentage of the control response for each subject (shown as dashed horizontal black line represent 100%). The asterisk above the post-conditioning bite force bar indicates that the reflex duration differs significantly from the pre-conditioned, * P = 0.003, (repeated measures ANOV with a post hoc Bonferroni adjustment).
5.4.4 Responses correlation

A Spearman's rank-order correlation was run to assess the relationship between EMG and bite force parameters. Preliminary analysis showed the relationship to be monotonic as assessed by visual inspection of a scatterplot.

There was a significant strong positive correlation between the integrals of the inhibitory waves in the ipsi- and contra-lateral EMGs and between bite force relaxation (as integral %ms) when analysing all sequences together. In addition, there was a significant small negative correlation between the size of the inhibitory wave in the ipsi and contralateral EMGs (as integral %ms) and the latency for bite force relaxation (Table 5-5).

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</thead>
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<td>0.624**</td>
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<tr>
<td>Bite force latency</td>
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<td>-0.241*</td>
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Table 5-5 A Spearman's rank-order correlation for main study variables, * = statistically significant at P < 0.05, ** = statistically significant at P < 0.000005
5.4.5 Conditioning procedure effect on pre-stimulus EMG frequency

As discussed in the introduction, the median power frequency in EMG spectrum would shift to lower frequencies in fatigued muscle (Figure 5-10). Therefore, the median power frequency was chosen to describe the power spectrum in the pre-stimulus EMG activity in all subjects. For the 300 ms pre-stimulus EMG in all experimental sequences, 512-point Fast Fourier Transformation (FFT) routine was carried out using Signal software to transfer the data from the time domain to the frequency domain and consequently calculate the median power frequency.

As shown in Figure 5-11, the average median power frequency for the 300 ms pre-stimulus ipsi- and contra-lateral EMG decreased from 153 ± 23.16 and 159.98 ± 26.75 Hz before the conditioning procedure to 145.95 ± 25.42 and 150.75 ± 23.37 Hz following the conditioning procedure. These decreases in the ipsi- and contra-lateral median power frequency of 4.79% and 5.24%, were statistically significant at P = 0.027 and P = 0.048, respectively (repeated measures ANOVA with a post hoc Bonferroni adjustment). Five and ten minutes following the conditioning procedure, there was an increase of the median power frequency level towards the preconditioning level such that there were no significant differences between the preconditioning and the 5 and 10 minutes post-conditioning sequences.
Figure 5-10 The power spectrums of 300 ms pre-stimulus contra-lateral EMG from one subject. Note the spectral shift to the left (towards lower frequencies) in the power spectrum of the post-conditioning (B) comparing to the pre-conditioning (A). As fatigue progresses there is a shift to lower frequencies where fast twitch motor units (higher frequency) drop out first and slow twitch motor units retained (lower frequency).
Figure 5-11 The median frequency of the EMG signals from ipsi- and contra-lateral masseter muscles. The asterisks above the bars indicate that the median power frequency differs significantly from the pre-conditioned latency, * P = 0.027, ** P = 0.048 (repeated measures ANOVA with a post hoc Bonferroni adjustment).
5.5 Discussion

5.5.1 General

The aim of this set of experiments was to investigate whether the mechanical manifestation of the inhibitory jaw reflex evoked by stimulation of the human lip, can be modulated by experimentally controlled conditions that mimic symptoms of a myogenous temporomandibular disorder. The results of this study are consistent with the results of Chapters 3 and 4 in demonstrating that the jaw inhibitory reflex is accompanied by a clear but proportionately smaller drop in forces between the teeth.

It has been shown previously, that the inhibitory jaw reflex responses to peri-oral stimuli are subject to modulation by heterotopic nociceptive stimuli and psychological factors (for review see Cadden, 2007). However, the effects of homosegmental conditioning stimuli on the jaw reflexes has been found to be substantial only when using more invasive techniques (Lobbezoo et al., 2006).

In these experiments under discussion, a non-invasive method was used to study the effect of a homosegmental conditioning stimuli on the mechanical properties of the inhibitory jaw reflex. This method, which consisted of an accelerated chewing task for 3 min, has been reported to cause a suppression in the jaw reflex under study (Maillou et al., 2010).

The conditioning procedure in this study (i.e. accelerated chewing task for 3 min) produced pain and/or fatigue in 17 out of 22 subjects. This is similar to previous
studies in demonstrating that jaw muscles pain and/or fatigue could be produced following chewing exercises for 3 min (Dao et al., 1994; Maillou et al., 2010).

5.5.2 Effect of the conditioning procedure

The induced pain and/or fatigue in this study produced an immediate depression of the inhibitory jaw reflex under study. This decrease in the response integrals and durations following the conditioning procedure was found to be significant in the bite force recording but not in the ipsi- and contra-lateral masseter EMGs when analysing the data from all subjects (n = 22). This lack of significant effects on the EMG-recorded inhibitory jaw reflex was somehow unexpected given the previous finding of the same conditioning procedure used in our lab where an accelerated chewing task for 3 min produced a significant suppression in the jaw inhibitory reflex (Maillou et al., 2010). It should be noted that there is inconsistency in literature concerning the effects of homosegmental, deep-somatic (e.g. Bendtsen et al., 1993; Truini et al., 2006; Maillou and Cadden, 2008) or cutaneous stimuli (e.g. Andersen et al., 1998b) on the inhibitory jaw reflex.

However, there was a statistically significant association between the occurrence of pain and/or fatigue and the changes of reflex size following the conditioning procedure. When analysing the data from subjects who reported pain and/or fatigue (n=17), there was a significant decrease in the reflex integrals for both EMGs and bite force recordings following the conditioning procedure. The duration of the responses
in the same group (i.e. those who reported pain and/or fatigue) was significantly decreased in the contralateral EMG and bite force recordings. The inhibitory reflex began to recover toward its pre-conditioned size and duration at 5 min following conditioning, and continued to do so at 10 min after conditioning.

The analysing of the pre-stimulus EMG frequency level in the different experimental sequences, revealed that there was a significant decrease in the pre-stimulus EMG median power frequency following the conditioning procedure, $P < 0.05$ (repeated measures ANOVA with a post hoc Bonferroni adjustment). This shift in the median frequency to lower frequencies is one of the well-known characteristics of a fatigued muscle (Stulen and De Luca, 1981). The results of the effect of the conditioning procedure used in this experiment, is similar to the findings of many other previous studies looking at the impact of the sustained clinching tasks (e.g. Lyons et al., 1993; Svensson et al., 2001).

Another effect of the conditioning procedure was also noted on the latencies of the responses. Following the conditioning procedure, there was a significant increase in the latencies of the inhibitory reflex in the ipsilateral EMG and bite force for all subjects. This finding was consistent with another study in our lab looking at the effect of the deep somatic afferent nerves in a remote part of the body on the inhibitory jaw reflex (Maillou and Cadden, 1997).

The delay in the reflex latency was also accompanied by a significant increase in the relaxation electromechanical delay (R-EMD). Previous studies on skeletal muscles other than jaw muscles have found the same effect following fatiguing tasks (Edwards
et al., 1972; Westerblad and Lannergren, 1991; Leung and Xiao, 1997; Cè et al., 2014).

5.5.3 Possible mechanisms

The modulation of the inhibitory jaw reflex by heterotopic stimulation is believed to be mediated by mechanisms acting on the reflex pathway at a pre-motorneuron level (Cadden, 2007). However, there is no clear evidence to suggest that the homosegmental effects, as seen in this study, would be mediated by the same pathways.

Analysis of the power frequency of the EMG spectrum for all subjects has shown that the conditioning procedure had a fatiguing effect on the masseter muscles. On the other hand, the effect of the conditioning procedure on the EMG-recorded reflex integrals was not significant (for the same subject group). From these findings, one could suggest that the homosegmental effect, that is seen in previous studies, is more likely to be related to pain rather than fatigue. However, such conclusion cannot be proposed unless the other studies had investigated the power frequency and found no evidence of fatigue. In an earlier study at our lab, Maillou et al. (2010) reported significant decrease in the reflex integrals following the conditioning procedure. However, about 40% of the subjects in that study reported pain and fatigue, while the same figure for the current study is 18%. This could also explain the difference in the results between the two studies.
Although there was a significant strong correlation between the integrals of the reflex in the EMG recordings and bite force relaxation, the effect of the conditioning procedure was more prominent in the bite force recording comparing to the masseter EMGs. The normalised bite force integrals and durations showed greater divergence from the controls following the conditioning procedure comparing to the normalised EMGs integrals and durations (Figure 5-5 and Figure 5-9). This could have contributed to the modulation of the inhibitory reflex in the other jaw-closing muscles. Because the bite force between the jaws is the collective force output of all the jaw-closing muscles, then any changes in the activity of any of these muscles could reflect on the bite force measurement. However, there is no evidence to suggest that the temporal muscles are more susceptible to the inhibitory reflex (Yemm, 1972b; Cadden et al., 1996).

The delayed latency following the conditioning stimulus is similar to what is seen in several neurophysiological studies (e.g. Hagbarth et al., 1995; Maillou and Cadden, 1997; Granata et al., 2004) when a procedure which is expected to inhibit a response, produces an increase in the latency of that response (inhibitory or excitatory responses). This is believed to be related – to a great extent - to the fact that the inhibition causes some point in the underlying pathway to take longer to reach threshold due to the shape of the excitatory postsynaptic potentials (EPSPs) (Schmidt et al., 1985).

In the present study, the increase in the relaxation electromechanical delay (R-EMD) following the conditioning procedure could be attributed to the increase in
intracellular [ADP] - resulting from ATP hydrolysis - which causes slowing of cross-bridges switch from a force-generating state to a non-force-generating state (Tesi et al., 2002; Poggesi et al., 2005). This finding supports the conclusion in Chapter 3 in that the rigor-like cross bridges are likely to be responsible, at least in part, for the R-EMD.
5.6 Conclusion

This study has shown that following an accelerated chewing task for 3 minutes, a significant decrease is seen in the integrals and durations of the mechanically-recorded inhibitory jaw reflex. In addition, there was a significant increase in the relaxation electromechanical delay following the conditioning procedure. The effect of the conditioning procedure was less differentiated in the EMG recordings comparing to the effect on bite force. The EMG responses showed a significant increase in the latency (ipsilateral EMG).

When analysing the data from the subjects who reported pain and/or fatigue (77% of the subjects), there was a significant decrease in the EMG and bite force integrals following the conditioning procedure. In the same subject group, the duration of the contralateral EMG decreased significantly following the conditioning procedure.

It can be concluded that the bite force recording (in adjunct to EMG) have provided stronger evidence than before that there might be loss of some of the "mechanical" protective effect of the inhibitory reflexes (against masticatory apparatus overload). The later could enhance our understanding of the jaw reflexes’ role in conditions such as TMD and chronic orofacial pain.
Chapter 6: General Discussion

6.1 Overview

As discussed in chapter 1, the studies described in this thesis were carried out to investigate the mechanical manifestations of the inhibitory jaw reflex that is evoked by electrical stimulation to the upper lip and the effect of experimentally-induced pain and/or fatigue on that jaw reflex. The main findings of the studies were:

1. The well-established inhibitory reflex, which can be seen in the EMGs of jaw-closing muscles following nociceptive stimulation of the upper lip, is accompanied by a clear, but proportionately smaller, drop in forces between the jaws. There are strong correlations between all the corresponding parameters (i.e. latency, duration, peak and integrals) of the inhibition waves in the EMG and force recordings.

2. The onset of the mechanical response is significantly delayed by comparison with the EMG response and lasts much longer.

3. When the duration of the EMG-recorded inhibitory reflex increases, there is a significant increase in the drop in the bite force.

4. Following a chewing task for 3 minutes which is usually fatiguing and/or painful, there is a significant decrease in the duration and size of the mechanically recorded reflex.

Thus, bite force recording (in addition to an EMG) provides additional evidence to suggest a protective effect - against masticatory apparatus overload - for the inhibitory
jaw reflex under study. It is clear that the inhibitory reflex recorded in the masseter EMG is, at least in part, responsible for the relaxation in the bite force. It is less likely that the jaw depressor muscles could have contributed to the drop in bite forces via an active opening reflex since such responses have been shown to require application of very high stimulation intensities (Cadden et al., 1997) and habituate very rapidly (Desmedt and Godaux, 1976; Cadden et al., 1997).

There was also a moderate negative correlation between the size of the EMG-recorded inhibitory wave and the latency in bite force with the size of the inhibition explaining 24% of the variation in the latency of the relaxation in bite force. These findings could be explained by the increased number of muscle fibres going into the fast exponential phase of relaxation, following the initial linear phase (Hoskins et al., 1999; Gordon et al., 2000; Tesi et al., 2002).

Following the fatiguing/painful chewing task, there were significant decreases in some of the parameters of the bite force recordings of the reflex. Indeed as reported in Chapter 5, the bite force recordings of the reflex appeared more susceptible to these conditioning effects than the accompanying EMG recordings. Additionally, there was a significant increase in the relaxation electromechanical delay (R-EMD) following the conditioning procedure. However, these effects were short-lasting with there being no significant effect on any of the reflex parameters 5 and 10 minutes following the conditioning procedure. Analysis of the power frequency of the EMGs in these experiments indicated objectively, a fatiguing effect on the muscle activity. However,
it is difficult to draw any conclusion as to whether the modulatory effect following the conditioning procedure resulted from pain, fatigue or even both.

Overall the results provide stronger evidence than before that there might be a loss of some of the mechanical protective effect of the inhibitory reflexes (against masticatory apparatus overload) as a result of homosegmental conditioning stimuli. It could be argued that this provides additional support for the theory that suggests a potential aetiological role for altered jaw inhibitory reflexes in the temporomandibular disorders (TMD) (Cadden and Orchardson, 2009). As discussed in Chapter 1, several groups have reported that inhibitory reflexes may be weaker (Maillou and Cadden, 2007) or absent (De Laat et al., 1985) in patients suffering from TMD. It is possible that some of the suppressed jaw inhibitory reflexes (loss in the negative feedback) in these patients could result in overuse of the jaw closing muscles and subsequently cause more pain in the muscles. There is no evidence to suggest whether the absence of the reflexes is a result or a cause of the clinical condition. Cadden and Orchardson (2009) suggested a vicious circle model in which symptoms of the TMD and the suppression of the reflexes may sustain each other (Figure 6-1).
Figure 6-1 Hypothetical relationship (vicious circle) between factors which could suppress inhibitory jaw reflexes and the symptoms of TMD. Pain and/or stress – both common symptoms of TMD – can cause a reduction in the jaw inhibitory reflexes. This in turn may cause an increased use of the muscles which may reinforce the pain felt from them. After Cadden and Orchardson (2009)
6.2 The Electromechanical Delay

The experiments in this thesis have shown that the timings of inhibitory reflex responses seen in force records can be significantly slower and longer-lasting comparing to those seen in the EMGs. More interestingly, the onset of relaxation in bite force following stimulation of the human lip lagged 13 milliseconds, on average, behind the corresponding reductions in EMG activity in the masseter muscle. Such a lag in jaw reflex relaxation time has previously been reported but not for this particular reflex. This relaxation electromechanical delay (R-EMD) was found to be in the range of 8 – 15 ms in the masseter muscle when the inhibitory reflex was evoked by intra-oral stimulation (Yemm, 1972a; Brinkworth and Türker, 2005; Yang and Turker, 1999). An R-EMD has also been noted in other human muscles (for the full list of studies see Table 3-4). Previous attempts were made to explain such a delay in the onset of the relaxation and have attributed it to the rate of Ca\(^{2+}\) reuptake by the sarcoplasmic reticulum rather than to myosin ATPase activity (Viitasalo and Komi, 1981a; Ce et al., 2013). However, the mechanisms behind the delay in the relaxation remain controversial. Indeed it may be more likely that rigor-state like cross-bridges are responsible for the delay and not the rate of Ca\(^{2+}\) reuptake.

Following the fatiguing and/or painful chewing task, there was a significant increase in R-EMD. Thus, it was clear that fatigue and/or pain may have altered the course of actions between the cessation of neuromuscular activity in the masseter muscles and the onset of the bite force decay. Similar effect following fatiguing tasks have been reported for skeletal muscles other than jaw muscles (Edwards et al., 1972;
Westerblad and Lannergren, 1991; Leung and Xiao, 1997; Cè et al., 2014). There is also some evidence in the literature to suggest that alterations in the delay are related to the rate of which cross-bridges switch from a force-generating state to a non-force-generating state during the first phase of relaxation (Tesi et al., 2002; Poggesi et al., 2005). The switching rate is believed to depend on the levels of free phosphate (Pi) and free adenosine diphosphate (ADP) in the muscle fibres (Hoskins et al., 1999).
6.3 Follow-on Studies

As mentioned above, it is not clear whether the modulation effect seen in the inhibitory reflex following homosegmental conditioning stimuli resulted from pain, fatigue or both. Similar effects have been reported in studies producing muscle fatigue only (van der Kaaij et al., 2009) and studies reporting muscle pain only (Wang et al., 1999). In addition to that, there is no enough evidence in the literature on how single motor units behave in the presence of muscle pain (e.g. Lobbezoo et al., 2002). It would be interesting to investigate this further by recording EMG from single motor unit rather than using surface EMG while providing (visual) feedback from the single motor unit recording. By providing a visual feedback from the single motor unit, one could instruct the subject to maintain same level of activity before and after the conditioning procedure (to compensate any fatigue effect) and see how that could be effecting the modulation of the inhibitory reflex seen in surface EMG following the conditioning procedure. Using this method along with bite force recording and conditioning procedure described in Chapter 5, could provide a greater insight into whether alterations of jaw motor function were related to pain and/or to fatigue.

As discussed in Chapter 3, the relaxation electromechanical delay (R-EMD), unlike the delay during excitation, has generated less interest and therefore the mechanisms behind it are not fully understood. In the course of the current study, it had been found that the jaw inhibitory reflex evoked by the electrical stimulation of the upper lip could provide an easy and controlled method to study the R-EMD under different physiological conditions. It would be interesting to investigate the effect of gender,
age, temperature and/or bite force level on the delay. This could provide more information on the behaviour of the jaw muscles during relaxation in human beings.

As established by previous studies in Dundee, the site and method of stimulation is important when investigating jaw reflexes (Cadden and Newton, 1988). It would be interesting to investigate the mechanical properties of different types of the inhibitory reflexes. Electrical stimulation across the periodontium is one appropriate method for evoking the type of reflexes which are likely to occur during normal function (Gardner et al., 2008). Comparing the mechanical manifestation of different types of jaw reflexes could help us understand the role of these reflexes and how they translate into overall loading changes between the jaws.

Finally, the general protocol and the bite force recording and analysing techniques used in this study could and arguably should, provide the basis for a clinical study on TMD patients. In that study, mechanical recordings of the inhibitory jaw reflexes would be carried out along with surface EMG recordings. It would be necessary to examine and accurately diagnose a group of TMD patients, using the internationally-accepted Research Diagnostic Criteria for TMD (RDC/TMD) method (Dworkin and LeResche, 1992). The reflexes would be recorded from this group and a group of age and sex-matched healthy controls. Further studies could be performed during and after the treatment of these patients.
References:


