The H-reflex in Human Masseter.

A thesis submitted

for the degree of

Doctor of Philosophy

in

The Department of Physiology,

The University of Adelaide,

Adelaide, South Australia.

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September 1999

CONTENTS

ABSTRACT

H-reflexes are used to determine the reflex connections of muscle spindle afferents, the excitability of the motoneuron pool and the integrity of the reflex pathways. However, H-reflexes are small and can be difficult to elicit in the masseter, limiting their use in the investigation of the masticatory system. H-reflexes may be difficult to elicit in masseter due to the nature of the input from the muscle spindles onto the motoneurons. Thus, this study investigated the recruitment of masseter motoneurons into the H-reflex, compared to the recruitment occurring during voluntary isometric biting, to determine the distribution of the effective muscle spindle input.

The depth of the masseteric nerve makes it difficult to stimulate using surface techniques, and inserted needle techniques may not be tolerated by patients or subjects. Therefore, the first part of this study describes a new transmuscular stimulating technique for masseter.

Previous studies investigating recruitment of motoneurons have relied on the use of spike-triggered averaging of force or spike amplitude as indicators of motoneuron size, but these techniques may not be reliable in masseter. The representation of a motor unit in the Macro EMG (MacroRep) has been shown to be appropriate as a measure of muscle unit size, so this was used in the current study to investigate the recruitment order of masseter muscle units during voluntary isometric biting. Recruitment of muscle units was shown to occur according to the size principle. The force recruitment threshold of masseter motor units was found to be much less stable than the surface EMG recruitment

threshold, probably due to the changing contribution of masseter, temporalis and digastric to the production of slow voluntary isometric biting ramps.

MacroRep amplitude has been shown to be an appropriate measure of muscle unit size, but the relationship between muscle unit size and motoneuron size has not previously been established in masseter. Therefore, to determine whether motoneuron recruitment, as well as muscle unit recruitment, occurred according to the size principle, the relationship between MacroRep amplitude (indicator of muscle unit size) and H-reflex latency (indicator of motoneuron size) was investigated. The strong relationship between these measures indicated that MacroRep amplitude may be used to indicate the relative sizes of motoneurons.

To determine the effectiveness of the Ia afferent input onto masseter motoneurons, H-reflexes were recording in simultaneously-active large and small motoneurons. H-reflexes were found to be more prevalent in large motoneurons than in small ones. This indicates that either Ia input is preferentially distributed to large motoneurons, or that the input to small motoneurons is pre-synaptically inhibited.

A more effective input onto large motoneurons suggests that the stretch reflex in masseter may have a limited role in the maintenance of static posture. The role of the masseter stretch reflex during mastication is likely to be for the development of large, fast forces when an unexpected resistance is encountered. More effective input onto large motoneurons would also allow for the development of large, fast bite forces when the gamma system is activated during attacking or fighting situations.

ACKNOWLEDGMENTS

There are many people I would like to thank for helping me during the work for this thesis.

First, my supervisor Dr Kemal Türker, who has been a source of continual support and encouragement, and who has been very generous with his time throughout the entire period of my candidature. I am grateful for his patience, flexibility to new ideas and directions, and interest in my project.

Thankyou to my husband Michael and my children Matthew and Claire for encouraging me and supporting me, and for helping me retain perspective when it all seemed too hard. Thanks also to my parents and friends who kept me going by their interest and support. Without the help of all these people, completing this project would have been far more difficult. Thanks to Dr. Brenton Dansie for assistance with statistical analysis. Thanks also to the other students in the laboratory, in particular Jun Yang, Susan O'Connor, Melissa Jenkins, Kylie Tucker and Peter Abolfathi, who were a great help in discussing ideas and perspectives, and who spent hours listening to me practice presentations.

Thankyou also to all the volunteers who took part in the study, without whom the study could not have occurred.

An Australia Postgraduate Award Scholarship provided financial assistance for the project, and I am grateful for this.

DECLARATION

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

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Chapter 1. Introduction and Aims

Headaches and facial pain resulting from abnormal function in the masticatory muscles (Bakke et al. 1996), occurs in up to 15% of the adult population (Bakke and Moller, 1992). It is generally accepted that prolonged, low level static activity or abnormal load in the masticatory muscles during chewing can result in pain (Rasmussen et al. 1977; Moller et al. 1979; Rugh and Solberg, 1979; Christensen, 1981). The masseter muscle makes a major contribution to the closing force of the mandible and thus can contribute to the development of pain in association with masticatory dysfunction (Bakke, 1993).

To enable symptoms of craniomandibular dysfunction to be treated appropriately, the mechanism of mastication, and the maintenance of normal jaw posture, need to be fully understood. To understand the complex movements of the jaw involved in chewing and speech, a detailed knowledge of the control of the masseter motor units by descending motor signals, and by feedback from sensory receptors in the muscles, gums, skin and joints is required (Miles, 1995). The jaw-closing muscles are richly supplied with muscle spindles, which are the sensory receptors for stretch and vibratory stimuli. Stretching the jaw-closing muscles, for example by applying a tap to the chin, stimulates the muscle spindles and results in a stretch reflex that can be recorded from the masseter using electromyography (EMG). Similarly, applying electrical stimulation to the masseteric nerve results in an H-reflex, due to direct stimulation of the afferent nerve fibres from the muscle spindles. Studying stretch and H-reflexes can provide information about the excitability of the motoneuron pool and about the central connections of various inputs

to the motoneuron pool. In the limb muscles, H-reflexes are often used in preference to stretch reflexes for this purpose, as the electrical stimulus can be more tightly controlled than the mechanical stimulus required for the stretch reflex, and the variability caused by alterations in the sensitivity of the muscle spindles due to gamma fibre activity can be eliminated. However, H-reflexes can be difficult to elicit (Godaux and Desmedt, 1975; Türker, 1978) and record (Macaluso and de Laat, 1995a) in the masseter, thus making this a limited tool to use for improving understanding of the reflex control of masseter.

Part of the reason H-reflexes may be difficult to elicit in masseter is due to the depth of the masseteric nerve. For this reason, techniques attempting to stimulate the masseteric nerve by applying electrodes to the skin surface require high stimulus intensities, which are uncomfortable for the subject and result in the stimulation of nerves other than the masseteric nerve. Techniques using monopolar or bipolar needles inserted near the masseteric nerve have been used effectively (Godaux and Desmedt, 1975; Macaluso and de Laat, 1995a and b),but displacement of the needles during jaw movements, and the necessity of repeated insertions to locate the best stimulating position, can limit the usefulness of these techniques. When eliciting H-reflexes in the limb muscles, the cathode component of the stimulus is usually placed on the skin over the nerve, while a larger anode is placed on the other side of the limb, thus the stimulating current passes right through the nerve. To achieve this in the masseteric nerve requires that the anode be placed inside the mouth. This type of stimulation has not previously been used to elicit masseter H-reflexes. Chapter 5 describes the development of such a stimulating technique.

Previous recordings of the H-reflex in masseter have relied upon surface or concentric needle electrode recordings, which show only gross representations of the reflex activity. In order to ascertain the effects of stimulation of muscle spindle afferents upon different motor units, recordings need to be made from individual motor units. Chapter 5 also describes this methodology, with results of H-reflexes in single motor units in masseter.

The difficulty of producing H-reflexes in masseter may in itself be suggestive of differences in reflex connections in this muscle. It is difficult to elicit H-reflexes in the small muscles of the hand (Mazzocchio et al. 1995), and it has been suggested that the reason for this is that the input from the muscle spindles, via the Ia afferents, may not be distributed onto the motoneurons in the same way as in other muscles. In most muscles, Ia afferent input is more effective on the small motoneurons (Awiszus and Feistner, 1995; Schmied et al. 1997). In the hand (Mazzocchio et al. 1995) and in tibialis anterior (Semmler and Türker, 1994), more effective Ia afferent input onto large motoneurons has been demonstrated. More effective afferent input from the muscle spindles onto the larger motoneurons makes H-reflexes difficult to record because the reflex response occurs in the axons of the large motoneurons, but these axons are the first to be blocked by antidromic impulses as a result of the stimulus. This could provide a possible explanation as to why H-reflexes are difficult to elicit in masseter. Thus, an aim of this study was to determine which motor units, the larger or the smaller, received more effective input from the muscle spindles. An H-reflex paradigm was used, and the

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presence of H-reflex responses was compared in concurrently firing large and small motor units. This is described in Chapter 8.

If the input from the muscle spindles is more effective on large motoneurons, this will mean that it will be the large motoneurons that are recruited first into the H-reflex. During normal isometric voluntary contractions, recruitment is generally believed to occur according to the size principle (Henneman, 1981a), so that motoneurons are recruited in order of increasing size. Due to the difficulties of directly measuring motoneuron size in humans, methods have been developed to measure the size of the muscle units. The size of the motoneuron is then inferred, as the number and size of the muscle fibres in a motor unit is a good indicator of motoneuron size (Buchthal and Schmalbruch, 1980; Henneman and Mendell, 1981b). Although previous studies have investigated muscle unit recruitment in masseter (Goldberg and Derfler, 1977a; Yemm, 1977a), the techniques used for determining muscle unit size were not appropriate (Hannam and Macmillan, 1994). An alternative method of determining muscle unit size has been described, where the contribution of a motor unit to the Macro EMG is determined (MacroRep) (Stålberg, 1980). This technique has been shown to be a reliable indicator of muscle unit size in the limb muscles and masseter (Stålberg et al. 1986; Dengler et al. 1989; Vogt et al. 1990; Lemon et al. 1990; Masakado et al. 1994). The MacroRep can be used to investigate the recruitment of muscle units into slow voluntary isometric contractions in masseter. This is described in Chapter 6.

In studies using MacroRep amplitude to determine muscle unit size, the assumption is made that small muscle units are supplied by small motoneurons, and large muscle units are supplied by large motoneurons. This is a reasonable assumption in the limb muscles, where small motoneurons supply thin Type I muscle fibres in small muscle units (small MacroRep amplitude) and large motoneurons supply a larger number of thicker Type II muscle fibres (large MacroRep amplitude). However, there is a complication when attempting to make the same inferences in masseter, as the Type I fibres are larger than the Type II fibres (Eriksson, 1982). Thus in masseter the muscle units with a smaller MacroRep may be those with a small number of large Type I fibres, or a large number of small Type II fibres. This has implications when using MacroRep to indicate motoneuron size and recruitment order in masseter. Therefore, the relationship between MacroRep amplitude and motoneuron size needs to be established for masseter. This was done by comparing H-reflex latency, an indicator of motoneuron size, with MacroRep amplitude, as described in Chapter 7.

Chapter 2. Masseter

2.1   Structure

Masseter is a complex muscle, which can be divided into deep, intermediate and superficial parts. The bulky superficial part arises from a thick, multileaved aponeurosis from the anterior two thirds of the lower border of the zygomatic arch and inserts from the angle of the mandible anteriorly to the ascending ramus. The intermediate part rises from the central, medial third of the zygomatic arch and the lower border of its posterior third, inserting on the central part of the ascending ramus. A deep part arises from the deep surface of the zygomatic arch and inserts on the upper part of the ascending ramus (Hannam and McMillan, 1994). Masseter is supplied by the masseteric nerve, which arises from the anterior branch of the mandibular nerve, passes anterior to the temporomandibular joint and enters masseter from its deep surface (Hannam and McMillan, 1994).

The internal architecture of masseter is complex. It consists of five internal aponeuroses that run parasagitally and attach to the zygomatic arch and mandible. The deep, intermediate and superficial parts of masseter are separated by the internal aponeuroses in the posterior part of the muscle, but the deep and superficial fibres fuse together in the anterior masseter (Zwijnenburg et al. 1999). Masseter is a multipennate muscle, with the different sets of muscle fibres aligned obliquely to one another. The pennation of the masseter muscle fibres allows large forces to be developed, with a relatively small range of movement (Hannam and McMillan, 1994; Weijs and Kwa, 1995). The length of

the muscle fibres ranges from 14 - 38 mm, with the longest fibres located anteriorly (van Eijden and Raadsheer, 1992). Most of the masseteric muscle fibres are short, and attach to the aponeuroses at acute angles (Schumacher, 1982). The fibre orientation of the masseter differs in the deep and superficial regions. In the superficial part, the fibres are more horizontal, converging from the mandibular angle to the anterior end of the zygomatic arch, whereas the deep fibres are more vertically oriented (Hannam and McMillan, 1994; Weijs and Kwa, 1995).

2.2 Functions of masseter

2.2.1 Maintenance of Mandibular Posture

Masseter is a major muscle contributing to the action of jaw-closing. Although the main function of the jaw-closing muscles is to enable the breakdown of food particles to a size that can be swallowed, they may also have a postural role, maintaining the mandible in a constant position in relation to the upper jaw during disturbing activities such as walking or running (Lund et al. 1983a). There is some dispute about the postural role of masseter. Eriksson et al. (1984) and Yemm (1977b) found no electromyographic (EMG) activity in masseter during the resting position of the jaw, in contrast to Goldberg and Derfler (1977a), who found that masseter was active during relaxed standing, and Lund et al. (1984) who found that masseter and temporalis were both active in stabilising the jaw in the cat. The use of EMG for studying the contribution of masseter to the control of posture may be limited by the effect of the recording electrodes on masseter activity, especially if intramuscular needles are used. Using

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surface electrodes to record electrical activity may be affected by cross-talk: Eriksson et al. (1984) found activity recorded by electrodes placed over masseter, in the mandibular rest position, was generated from muscles with facial nerve innervation. Additionally, due to the anatomical complexity of masseter, it may be that some parts are active in postural control while others are not. Some authors (Yemm, 1977b; Miralles et al. 1987) have considered that most postural control is provided by anterior temporalis, with masseter providing the force required for mastication. Lobbezoo et al. (1993a) compared the sensitivity, in terms of gain and threshold, of the jaw-jerk responses in masseter and temporalis and found that they were similar, therefore concluding that masseter and temporalis could both have a role in the maintenance of jaw posture. However, the presence of stretch reflexes in a muscle may not necessarily imply that the role of these stretch reflexes is for the maintenance of posture (Dessem, 1995).

The low levels of activity recorded in the jaw muscles during upright posture (Yemm, 1976; Rugh and Drago, 1981; Miller, 1991) may indicate that passive tension in the jaw-closing muscles provides the support for the jaw. The contribution of passive tension in the jaw closers to maintenance of mandibular posture in the upright position was investigated by Lanenbach and Hannam (1999) using a dynamic model of the jaw. These authors found it unlikely that passive tension in the jaw closers was solely responsible for the maintenance of jaw posture, indicating that some activity in the jaw closers was required.

2.2.2 Jaw Closing

Masseter is only one of the muscles producing closing of the jaw, the other major jaw closers being temporalis and medial pterygoid (Warwick and Williams, 1995). The major antagonist to jaw-closing is the digastric muscle (Warwick and Williams, 1995). The relative contribution of masseter to the production of jaw-closing force has been shown to be dependent on the direction of the biting (van Eijden et al. 1990; Hickman et al. 1993). Van Eijden et al. (1990) investigated recruitment activity of masseter, temporalis and digastric during biting in different directions. Significant differences in the activity of posterior temporalis and masseter were recorded with differences in bite direction, however there were very small changes in the activity of anterior temporalis with changes in biting direction. Although the results of the seven subjects in this study were considered similar, and were subsequently pooled, considerable differences in the activity of the jaw-opening and closing muscles can be observed, even when the direction of biting was the same (Figure 1). For example, when biting at 350 N, the contribution of left anterior masseter varied between 20 and 70% of maximum masseter EMG in different subjects. Differences in biting patterns between subjects may be due to differences in jaw architecture (Hickman et al. 1993). The study by van Eijden et al. (1990) investigated biting patterns in 50 N force increments, with no measurements made of the first 50N. Although force-EMG relationships were considered to be linear in this study, steep increases in anterior temporalis activity must have occurred during the first 50N, as the force-EMG regression line did not pass through zero. Thus the recruitment patterns that subjects use to develop the first 50N of force cannot be concluded from this study, nor from a similar study by Mao and Osborn (1994) which

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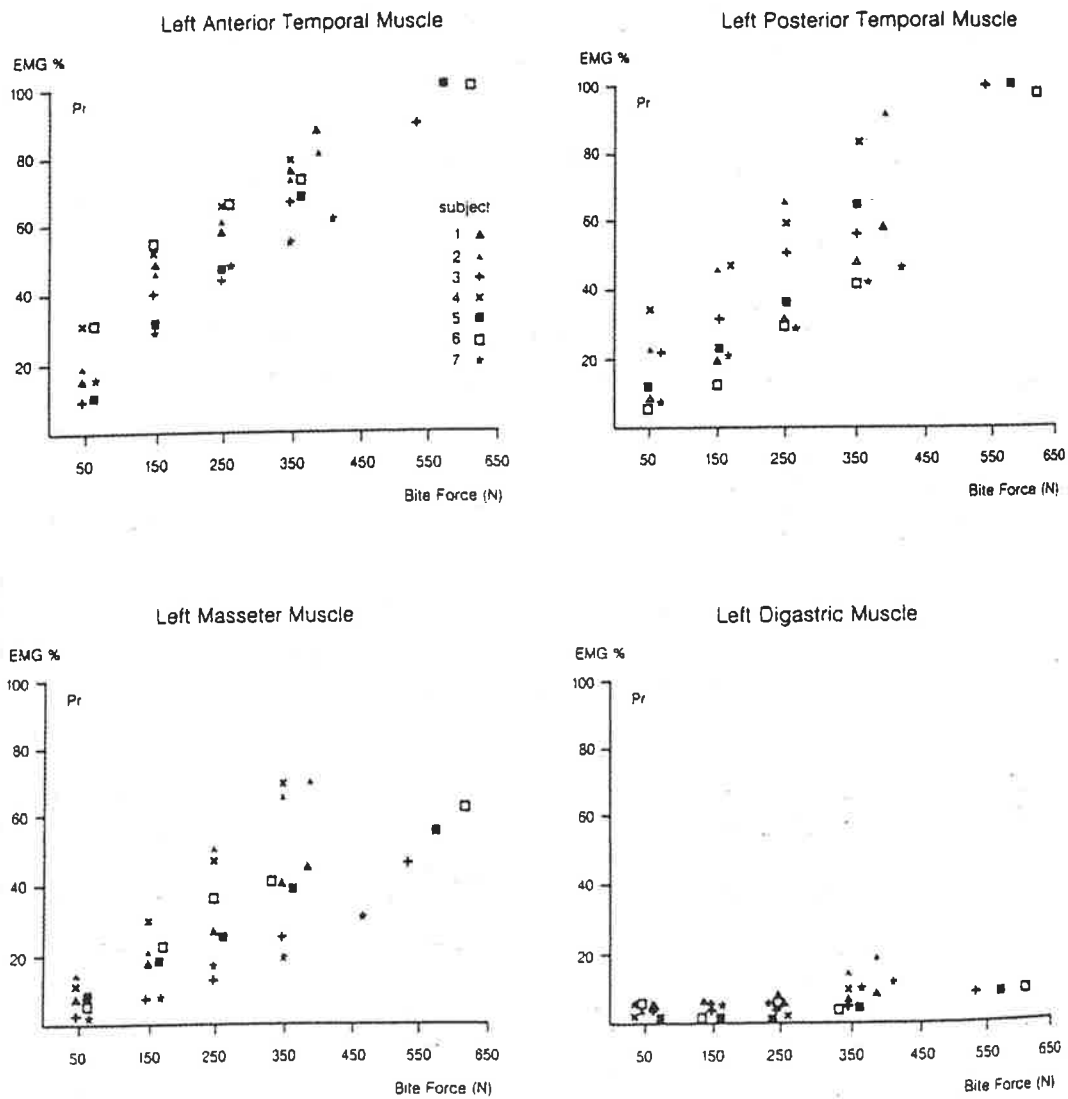
Figure 1. Contribution of Masseter, Temporalis and Digastric to Biting.

Bite force-EMG plots of the left sided jaw-closing and jaw opening muscles, during a medially-directed bite recorded at the right premolar (Pr). The results for seven subjects are presented, showing that there were quite different patterns used by these subjects. Reproduced from van Eijden et al (1990).

also used 50N increments to investigate the contribution of masseter and temporalis to biting. That study found that subjects used similar ratios of masseter/temporalis activity when biting direction was the same. Blanksma et al. (1995) alluded to the possibility of greater variation in the contribution of the jaw-closing muscles at lower force levels, since at higher force levels the number of different neural strategies for force development decreases.

Thus, although masseter has a role in closing the jaw, its contribution may change with the direction of biting. Questions also remain about the stability of the recruitment patterns of the jaw-closing muscles used by subjects during the development of biting force. The stability of the recruitment patterns of the jaw-closing and opening muscles has not been investigated for individual subjects. Strictly controlling the bite force direction by using triaxial force feedback as used by van Eijden et al. (1990) may overcome the impact of varying biting directions on the contribution of the jaw-closing muscles, but cannot control the effect of varying activity of digastric, which may co-contract during jaw closing. The biting patterns used during the development of the first 50N warrant investigation, especially as these are the bite forces used most frequently during normal mastication (Anderson, 1956; Richter, 1995).

2.3 Motor units and muscle fibres.

Every muscle fibre is innervated by only one motoneuron, but each motoneuron supplies a number of skeletal muscle fibres. The motoneuron, its axon and all of the muscle fibres

it supplies is called a motor unit (Sherrington, 1929). The motor unit represents the smallest functional unit available for muscle activity. At each discharge, all muscle fibres in a motor unit are activated, in the "all-or-nothing" twitch response. Burke (1973) described the "muscle unit", the force-producing part of the motor unit, as consisting of all the muscle fibres supplied by a motoneuron.

Muscle units have different colours and behave in different ways. Light coloured muscle units contract rapidly and fatigue quickly, whereas red motor units contract slowly and fatigue slowly. These differences between motor units are due to different histochemical properties of the muscle fibres of which they are comprised, and different characteristics of the motoneurons supplying them. Functionally, there are three classes of motor units (Burke et al. 1973). The first group contracts and relaxes rapidly, and generates high force levels, but fatigues quickly. These units are known as fast fatigable (FF) motor units. The second group is known as slow (S) motor units as they contract and relax slowly. They only produce about 10% of the force of the FF units, but they are very fatigue-resistant. Type S units usually have the lowest recruitment thresholds whereas FF motor units are recruited at higher force levels (Eriksson, 1982). FF motor units produce much more force output than S motor units, as the innervation ratio (the number of fibres in the motor unit) is higher, and each of the muscle fibres produce higher force levels, due to their greater cross-sectional area (Ghez, 1991). The third group fall between FF and S units, in that they contract and relax nearly as rapidly as the FF units but are much more fatigue-resistant (fast, fatigue-resistant or FR units: Burke et al. 1973).

There are three main types of muscle fibres found in skeletal muscles, and their distribution is usually tightly correlated with the type of motor unit to which they belong. Thus, S motor units consist of fibres that contract and relax slowly, contain large amounts of myoglobin and are dependent on oxidative metabolism. These are termed Type I fibres. The Type I fibres are more commonly found in the deeper layers of the muscle, where the oxygen supply is richest (Ghez, 1991). FF motor units consist of fibres that are fast and rely on glycolysis for energy production. These fibres produce high force levels but fatigue quickly and are termed Type IIB fibres. They are larger in diameter than the Type I fibres. FR motor units consist of Type IIA fibres, which are rapidly contracting but more fatigue resistant, using glycolytic and oxidative measures for energy· production (Buchthal and Schmalbruch, 1980). Type IIC fibres have characteristics between Type I and Type II fibres. They are normally rare in human limb and trunk muscles (Häggmark and Thorstensson, 1979) but are seen during muscle development in conjunction with physical training (Jansson et al. 1978).

The motoneurons supplying the motor units demonstrate characteristics supportive of the function of the motor units. Type S motor units have small, high resistance motoneurons with slowly conducting axons. Type FF motor units have large, low resistance motoneurons with rapidly conducting axons. Thus, the speed of contraction of the muscle fibre is related to the speed of action potential conduction along the axon of the motoneuron supplying the motor unit.

Carlsöö (1958) calculated mean values of motor unit size in masseter, and estimated that there were 1452 motor units in human masseter, with a mean number of 640 fibres per unit. This compares to 209, 329 and 239 fibres per motor unit for biceps brachii, tibialis anterior and deltoid (Gath and Stålberg, 1981). The range in the number of fibres per motor unit in masseter is not known, but it has been argued that there should be a large range (Stålberg et al. 1986). From low-threshold units, Stålberg et al. (1986) estimated that small units would contain 100 muscle fibres or less. Comparing this to Carlsöö's (1958) mean value from all units of 640 fibres per unit, suggests a large range of sizes. A wide range in the number of fibres per motor unit has also been found in limb muscles in cat gastrocnemius (Burke and Tsairis, 1973; Rafuse et al. 1997) and human hand muscles (Feasby and Brown, 1974).

Individual muscles contain varying proportions of motor unit types, and in the limb muscles the fibres of a motor unit are spread widely throughout the muscle. Human jaw muscles have a specialised muscle fibre composition, different from that of limb muscles and probably related to their unique function (Stålberg et al. 1986). There are large groups of densely-packed fibres of the same histochemical type, a finding which would indicate re-innervation if present in the limb muscles. However, although there is clustering of fibre types, these fibres are from different motor units (Stålberg et al. 1986).

In masseter, Type I muscle fibres are predominant throughout, except in the posterior superficial portion where Type I and Type IIB fibres occur with similar frequencies. In

the anterior portion (deep and superficial layers), Eriksson (1982) found that Type I fibres account for about 70% of all fibres. This is in contrast to Tuxen et al. (1999) who found a greater proportion of Type II fibres in superficial anterior masseter. The reason for these different findings is not clear, although Eriksson (1982) used autopsy material from the entire masseter whereas Tuxen et al. (1999) used material from a single biopsy of superficial anterior masseter, from subjects undergoing extraction of the third molar. A high proportion of Type I fibres suggests that masseter will be highly resistant to fatigue, and that most units will be activated at low forces, allowing more precise control over masticatory forces and the maintenance of jaw posture (Mao et al. 1992). Posterior superficial masseter has the highest percentage of Type IIB fibres, suggesting a capacity in this part of the muscle for higher muscular tension but with fatigue occurring more quickly. There is a very small population of Type IIA fibres in human masseter. There is however a moderate population of Type IIC fibres, about 5-10% of all fibres (Eriksson, 1982). These fibres would be considered transitional if they occurred in the limb muscles (Rowlerson, 1990). It is not known whether the jaw muscles are in a continual state of transition or if these fibres form a stable population.

As masseter is primarily composed of Type I fibres, it would be expected that there would be a large number of Type S motor units. Nordstrom and Miles (1990) found a poor correlation of motor unit fatigability with motor unit twitch amplitude and contractile speed, as measured by spike-triggered averaging of force with the firing of a motor unit. On this basis, these authors suggested that there was only a small population of Type S (fatigue-resistant) motor units in masseter. This would suggest that the

correlation between the histological characteristics of motor units and their physiological properties is not strong in masseter. However, these findings must be interpreted with caution, as the use of spike-triggered averaging for eliciting twitch characteristics is potentially unreliable in masseter (Hannam and McMillan, 1994) (see Section 2.6.2.1).

In the limb muscles, the IIA and IIB fibres are larger than the Type I fibres. This is not the case in masseter, where except for in the intermediate portion, the Type I fibres are larger than Type II fibres (Eriksson, 1982). This may be due to the lack of exercise in the masseter, due to the soft refined foods in the modern diet (Eriksson and Thornell, 1983). However, as pointed out by Lobbezoo et al. (1993a), the limb muscles of modern man are not required to perform the heavy loads needed in previous years, but the relative diameters of the Type I and Type II fibres are maintained.

2.4   Functional Compartmentalization

2.4.1 Physiological Basis in Masseter

In muscles with neuromuscular compartments, the primary branch of a muscle nerve innervates separate portions of the muscle almost exclusively. The separate parts of the muscle may therefore be activated independently, contributing to different motor tasks. There are three major nerve branches to masseter, supplying the anterior, inferolateral and deep regions (Xiguang et al. 1986). These three major branches divide into between 11 and 13 primary branches (in the rabbit, Widmer et al. 1995) allowing for the presence of neuromuscular compartments.

In the rabbit masseter, clear separation of the masseter in anatomical compartments has been demonstrated. The superficial masseter can be divided into three compartments and the intermediate layer is composed of two layers. The posterior deep layer has only one compartment, while the anterior deep layer has four parts which are separated by connective tissue and nerve branches (Widmer et al. 1995). These authors concluded that the complex pattern of anatomical partitioning, supplied by different nerve branches, provided the basis for individual activation of distinct areas of masseter.

Glycogen depletion techniques in the rabbit (Weijs et al. 1995; Weijs and Kwa 1995) and scanning EMG (Tonndorf and Hannam, 1994) and stereotactic imaging (McMillan and Hannam, 1991) in humans, have shown that most motor units are confined to small areas within compartments of the masseter muscle. In the rabbit masseter, which is very similar to the human masseter, motor units occupied an area of only $7.9mm^2$ (Weijs and Kwa, 1995) and showed large differences in fibre composition and action lines depending on their location in masseter (Turkawski et al. 1998). This is in contrast to the limb muscles where motor unit fibres are usually distributed through a larger area within a compartment (English and Weeks, 1984). The small territories of the motor units enables small areas of the muscle to be activated in a selective fashion, supporting the ability of different compartments of masseter to contribute to different activities (Eriksson et al. 1984; Turkawski et al. 1998).

Spatial separation of the motoneurons supplying deep and superficial masseter would suggest that the different parts are differentially activated. Whether or not the motoneurons are organised according to the area of masseter they supply is controversial. In the rabbit (Weijs and Kwa, 1995) and the monkey (Mizuno et al. 1981), the motoneurons of deep masseter have been found to be spatially separate from those of the superficial masseter. However, Widmer et al. (1995) and Saad et al. (1997) found that there was no anatomical partitioning of masseter motoneurons, so that differential activity of the compartments in masseter was not due to spatial coding of the motoneurons.

2.4.2 Functional Compartments in Masseter

Three functional parts have been demonstrated in masseter: anterior deep, posterior deep and superficial (Blanksma et al. 1992; van Eijden et al. 1993; Blanksma et al. 1995). In deep anterior masseter, the units mainly contribute to coactivation of the jaw muscles with the jaw slightly open, whereas deep posterior masseter is most associated with teeth clenching in the intercuspal position. Blanksma et al. (1990) showed that the posterior deep masseter acted more like part of temporalis, probably due to the vertical orientation of the muscle fibres. The fibres in the most posterior part of the deep masseter pass so close to the axis of the temporomandibular joint that their function as jaw closers is probably very limited (Weijs and Kwa, 1995). In the posterior superficial part, most motor units are involved in tooth contact tasks, with little or no role in maintaining jaw posture (McMillan and Hannam, 1992). These studies investigated

voluntary activation of masseter during static (Blanksma et al. 1992) and dynamic (van Eijden et al. 1993; Blanksma et al. 1995; Blanksma et al. 1997) biting tasks. The evaluation of the activity of a single part of a muscle is not possible during voluntary contractions, as other parts of the muscle will contribute to the motor tasks. To overcome this problem, Zwijnenburg et al. (1999) used monopolar stimulation of anterior, middle and posterior superficial masseter, and deep masseter, to investigate compartmentalised function in masseter. They found that stimulation of deep masseter caused mainly lateral and vertical movement of the mandible, whereas all three parts of superficial masseter produced anterior and vertical movement of the mandible. This study was limited by the use of monopolar stimulation of the different sections of masseter, which may have resulted in stimulation of sections other than where the electrode was inserted (the position of the indifferent electrode is not described). However, the results do support the division of masseter into deep and superficial parts, but not further subdivision of the superficial part into anterior, middle and posterior parts. The posterior part of deep masseter was found to produce movements similar to those produced by anterior temporalis suggesting that the deep masseter and anterior temporalis work together in the maintenance of mandibular posture (Zwijnenburg et al. 1999). Whether the anterior and posterior parts of deep part of masseter produce different movements was not established in this study, as the methodology only allowed for the placement of stimulating electrodes in the posterior part of deep masseter.

2.5 Muscle spindles in masseter

Muscle spindles are elongated structures, usually located in the deep part of a muscle, running parallel to the muscle fibres. Their size ranges from 4 to 10 mm in length. The three main components of muscle spindles are the intrafusal muscle fibres, sensory endings and motor (gamma) axons. The intrafusal muscle fibres are smaller than normal muscle fibres, and their central regions are non-contractile (Berne and Levy, 1985). Intrafusal muscle fibres may be nuclear bag or nuclear chain fibres, depending on the distribution of the nuclei. In most mammalian muscle spindles there are two nuclear bag fibres, one static and one dynamic, and several nuclear chain fibres (Sicher and DuBrul, 1975). Muscle spindles are innervated by Group Ia and Group II afferent fibres (Ghez, 1991). The Ia afferent endings (annulospiral endings) wind around both types of intrafusal fibres, and are stimulated by stretch applied to the intrafusal muscle fibres. Secondary endings of Group II afferents end mainly on nuclear chain fibres, in "flower-spray" endings. The primary spindle endings are phasic in behaviour, firing at high frequency and showing activity during jaw opening and silence during closing. The primary endings are rate-sensitive, so they fire at higher rates when length is changing than when length is maintained. In contrast, the Group II (secondary) endings fire tonically, at a lower frequency, and fire throughout the chewing cycle (Taylor et al. 1976). The intrafusal muscle fibres are supplied by gamma motoneurons. Excitation of the gamma motoneurons causes increases in firing of the muscle spindles due to contraction of the poles of the intrafusal muscle fibres and hence stretch applied to the central non-contractile area (Ghez, 1991). In this way, the sensitivity of the muscle spindles can be adjusted.

The jaw-opening muscles do not contain muscle spindles (Taylor et al. 1976) but the deep and anterior areas of masseter are richly supplied (Rowlerson, 1990). Estimates of the number of muscle spindles in cat masseter range from 34 (Lund et al. 1978) to 101 (Burhanudin et al. 1996). The muscle spindle count by Lund et al. (1978) may have been lower as these authors detached the muscle from the bone, possibly resulting in destruction of some spindles, whereas Burhanudin et al. (1996) kept the attachments intact. In masseter, most muscle spindles are located in the deep part of the muscle, in animals (Maier, 1979; Rowlerson et al. 1988; Sciote, 1993) and humans (Eriksson and Thornell, 1987). Thus, the muscle spindles are in close association with the areas of masseter that contain a high proportion of Type I fibres (Burhanudin et al. 1996). There are very few muscle spindles in superficial masseter (Rowlerson et al. 1988).

Masseter muscle spindles are larger and more complex than muscle spindles in limb muscles. They are commonly clustered, sometimes sharing capsule tissue (Eriksson and Thornell, 1987). Muscle spindles with up to 36 intrafusal fibres grouped into separate bundles have been found in masseter (Eriksson et al. 1994). The reason for the complexity of the masseter muscle spindles is not clear.

2.5.1 Projection of muscle spindle afferents onto motoneurons

The cell-bodies of the masseter muscle spindle afferents are located in the mesencephalic trigeminal nucleus, and processes from the cell bodies make monosynaptic contact with

jaw-elevator motoneurons (Taylor et al. 1995). Using succinyl-choline (SCh) to classify spindle endings, Taylor et al. (1995) showed that the projection to the trigeminal motor nucleus (Vmo) was primarily from the primary (Ia) afferents, whereas the secondary (II) afferents mainly projected to the supratrigeminal region. In the cat masseter, both primary and secondary afferents project monosynaptically onto homonymous motoneurons, although the input from the secondary afferents is only 70% of that from the primary afferents (Appenteng et al. 1978).

In the limb muscles, virtually all motoneurons in a pool receive short-latency excitatory inputs from individual homonymous muscle spindle primary afferents (Mendell and Henneman, 1971). Projection rates from 80 - 100% have been found in animals, depending on the preparation used (intact anaesthetised or spinal preparations: Nelson et al. 1979). Similar high projection rates are found in most human limb muscles (Buller et al. 1980; Mao et al. 1984). In some limb muscles this pattern is not present. For example, low projection frequencies have been found in cat neck muscles (10%: Keirstead and Rose, 1999) and external intercostal muscles (42%: Kirkwood and Sears, 1982). In the rat, each spindle afferent projects onto only about 10% of homonymous masseter motoneurons (Appenteng, 1978; Dessem and Taylor, 1989; Grimwood and Appenteng, 1995), possibly because the afferents project only onto motoneurons with a similar mechanical function (Appenteng et al. 1978). Thus, the Ia afferents may only project onto motoneurons supplying the same muscle compartment, a concept known as partitioning (Vanden Noven et al. 1986; Windhorst et al. 1989), which has been demonstrated in the cat.

There is also some evidence for a limited projection of Ia afferents in the human masticatory system. Miles et al. (1995) investigated the responses of human masseter motor units to stretch, and found that 35% of motor units did not have a significant short latency response. They suggested that non-uniform distribution of Ia input or pre-synaptic inhibition of Ia input may be responsible for this finding. They found no relationship between the discharge rates of the motor units and the size of the short-latency reflex response, suggesting that the distribution of Ia afferents onto motoneurons was not systematically organised according to size. However, all units were recruited at less than 15% of maximum voluntary contraction, so there were not large differences between the recruitment thresholds of units in a pair. Macaluso and de Laat (1995a) suggested that low projection rates of Ia afferents onto masseter motoneurons was responsible for the small size of the H-reflex (compared to the M-wave) in masseter. Lobbezoo et al. (1993a) suggested that Ia afferents in masseter projected only onto the motoneurons supplying motor units in deep masseter, as this was where most reflex activity occurred. However, these findings were based only on recordings from surface electrodes, which does not allow for accurate determination of motor unit location. The question of the distribution of Ia afferents onto masseter motoneurons in humans thus warrants further investigation, using a larger range of motor unit sizes, and recording from motor units whose relative sizes have been accurately determined.

2.6 Development of Force in Masseter

The increase in force during a slowly increasing isometric contraction can occur either by the recruitment of new motor units (recruitment) or by increasing the firing frequency of the motor units already firing (rate coding) (Kernell and Sjoholm, 1975; Nordstrom and Miles, 1991b). Small muscles, like those in the hand, tend to rely on recruitment at low force levels, but then use rate coding to increase force above about 50% of maximum force (Kukulka and Clamann, 1981). Masseter has been shown to use both recruitment of new motor units and rate coding of units already firing to increase interocclusal force (Derfler and Goldberg, 1978). However, as over 50% of masseter motor units are recruited at forces less than 4kg, this suggests that masseter is primarily a rate-coding muscle (Goldberg and Derfler, 1977a).

2.6.1 Firing frequency of masseter motor units

As described above, when the force developed by a muscle is increased, the firing frequency of recruited motor units increases. Erim et al. (1996) described the "onion skin" phenomenon, whereby at any given point in a ramp contraction, the earlier-recruited motor units fire at a higher frequency than later-recruited motor units. However, the earlier-recruited motor units start their firing at lower frequency than the later-recruited units. The lower firing frequencies of the higher threshold units have the advantage of minimising fatigue. Even though higher-threshold motor units begin firing at higher rates than the lower threshold motor units, at the instant of their recruitment,

as in every other moment of the contraction, their firing rates are lower than the firing rates of the lower threshold units.

In masseter, the relative firing frequencies of pairs of units during a steady contraction is used to reflect their recruitment order, with the unit recruited at a lower force level assumed to have the higher firing rate (Derfler and Goldberg, 1978; Nordstrom and Miles, 1991b). When comparing firing rates of motor units at different contraction speeds, Masakado et al. (1995) found that while firing rates increased with faster contraction, a high threshold motor unit always had a lower firing rate than a lower threshold one. However, Nordstrom and Miles (1991b) found that during long contractions in masseter the initial rate of firing of a motor unit may increase or decrease as force increased, and that this change does not depend on the initial firing rate of the unit. Thus using firing rate may not be a useful indicator of motor unit size in masseter during prolonged contractions.

2.6.2 Recruitment and the size principle

Recruitment of new motor units into gradually increasing contractions occurs according to the size principle, originally described by Henneman et al. (1965). According to the size principle, it is the size of the motoneuron that determines its place in the recruitment order. Thus:

"The amount of excitatory input required to discharge a motoneuron, the energy it transmits as impulses, the number of muscle fibres it supplies, the contractile properties of the motor unit it innervates, its mean rate of firing and even its rate of protein synthesis are all closely correlated with its size" (Henneman, 1977 p50).

Recruitment order is not solely dependant upon motoneuron size, as different synaptic inputs may change the recruitment order. This is further discussed in Section 2.6.3. When motoneuron recruitment occurs according to the size principle, it is the small type S motor units that are recruited first, followed by FR and FF motor units (Burke, 1981). Recruitment order organised by motor unit size has the advantage that the most frequently used units are those that are smaller and are most resistant to fatigue. The gradual recruitment of slow units allows for a smooth increase in force (Cope and Pinter, 1995) and motoneuron recruitment according to size relieves the central nervous system of a vast computational task in determining which motor units need to be recruited next.

The intrinsic excitability of a motoneuron is capable of determining recruitment order according to the size principle (Cope and Pinter, 1995). A range of factors are responsible for the intrinsic excitability of a motoneuron, including the specific membrane resistivity, cell surface area and voltage threshold of the cell (Gustafsson and Pinter, 1984; Heckman and Binder, 1990).

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To determine whether the recruitment of motoneurons occurs according to the size principle, it is necessary to be able to measure relative motoneuron size. Estimation of motoneuron size is necessarily indirect in humans, but the number and size of the muscle fibres of a motor unit (muscle unit) is a good indicator of the size of the motoneuron innervating these muscle fibres (Buchthal and Schmalbruch, 1980; Henneman and Mendell, 1981b). Therefore, most techniques utilise measurements of muscle unit size as indicators of motoneuron size. Various methods have been developed to indicate the relative sizes of muscle units. Using these techniques, muscle unit recruitment has been shown to be orderly according to size in the hand muscles (Milner-Brown et al. 1973b; Thomas et al. 1987), deltoid (de Luca et al. 1982), flexor carpi radialis (Calancie and Bawa, 1985), tibialis anterior (Desmedt and Godaux, 1978a), masseter (Goldberg and Derfler, 1977a; Yemm, 1977a; Desmedt and Godaux, 1978a; Desmedt and Godaux, 1979) and vastus lateralis (Cremer et al. 1983). Recruitment has been shown to occur according to the size principle during isometric contractions (Thomas et al. 1987) and during ballistic and vernier movements (Desmedt, 1983). In muscles with several compartments, recruitment occurs according to the size principle within a "task-specific" sub-population of the motoneuron pool (Calancie and Bawa, 1990). These studies used a variety of techniques to determine muscle unit or motoneuron size. The strengths and weaknesses of the methods for determining muscle unit size or motoneuron size are discussed below.

2.6.2.1 Spike triggered averaging of force.

Large motoneurons innervate muscle units that contain many fibres. These larger muscle units develop higher mechanical forces. Thus, the relative sizes of the muscle units, and their motoneurons, can be determined from the forces they develop. To determine the contribution of a muscle unit to the force output of a muscle, spike triggered averaging (STA) is used (Stein et al. 1972). In this technique, the force record from a muscle is averaged, with the sweeps of the averaging being triggered by the firing of a motor unit. It is assumed that the averaged force is an accurate measure of the muscle unit's tension because other units usually fire asynchronously with the unit of interest, especially at low force levels.

Orderly recruitment of muscle units in humans was first demonstrated in the first dorsal interosseous of the hand using the STA technique by Milner-Brown et al. (1973b). A positive linear relationship was found between the twitch tension developed by the muscle and the force level at which it was recruited during slow isometric contractions. Yemm (1977a) used spike-triggered averaging of force to investigate the twitch-tension of progressively recruited units in human masseter, and found that muscle units were recruited in order of increasing twitch tension.

There are limitations to the STA technique (Calancie and Bawa, 1990), particularly in the jaw muscles, as the STA technique does not take into account the lever length and the direction of force vector for each motor unit. The use of STA to measure twitch tension in masseter motor units is extremely difficult, due to the complex internal

architecture of the masseter. The twitches generated by a jaw muscle will not necessarily be reflected in the force measured when the transducer is placed between the teeth (Hannam and McMillan, 1994). Twitch tensions recorded with a force transducer placed between the teeth must always under-represent true tension, as the masseter is located between the temporomandibular joint and the tooth contact point, with the jaw behaving as a loaded beam in these conditions (McMillan et al, 1990). The twitch tensions calculated by STA vary according to the bite point angle of the force transducer used (McMillan et al. 1990). Additionally, STA records are contaminated by the presence of co-contraction of the jaw openers and closers which occurs during most functional activities (van Eijden et al. 1990). Goldberg and Derfler (1977a) describe the practical difficulties of extracting twitch characteristics from STA of force: the signal-to-noise ratio becomes very high with units producing low force levels, the high firing frequency of these units (even close to their threshold) results in fusion of the twitches, and peak tension is difficult to determine.

Spike-triggered averaging is also used to provide information about the time-course of the muscle unit twitch. In the limb muscles, there is a strong relationship between recruitment order and twitch contraction time, with the slower units being recruited first (Rowlerson, 1990). This pattern has not been found in the jaw muscles (Goldberg and Derfler, 1977a; Yemm, 1977a). The reason for this lack of relationship is not clear, but it may be due to inadequacies in the STA technique. Alternatively, it has been suggested that it could be due to the small size of the Type IIB fibres in masseter (Eriksson et al. 1994). These small muscle fibres may be contained in the smaller motor units, and could

thus be recruited first. However, this is contrary to the relationship between muscle unit size and muscle fibre type found in the limb muscles (Milner-Brown et al. 1973a).

2.6.2.2   Force recruitment threshold

If motor units are recruited in order of increasing size, then the force level at which a unit is recruited could indicate the relative sizes of the units. However, force recruitment thresholds have been found to be unstable in biceps brachii (Suzuki et al. 1990) and masseter (Hannam and McMillan, 1994). Factors such as the contraction history of the muscle and the changing activation of synergists and antagonists will affect the force recruitment threshold of a motor unit. In masseter, the amount of jaw opening dramatically affects the force recruitment threshold of motor units, making this an inadequate criterion for determining motor unit size (Miles et al. 1986). Speed of contraction (Desmedt and Godaux, 1977) and fatigue (Nordstrom and Miles, 1991b) also affect recruitment thresholds in the jaw muscles.

As described by van Eijden et al. (1990), the contribution of masseter to jaw-closing depends on the direction of biting used. Thus the force recruitment threshold of masseter motor units will also be affected by the direction of biting used.

2.6.2.3   Spike amplitude

Goldberg and Derfler (1977a) studied the relationship between spike amplitude (bipolar recording of single motor unit activity), twitch tension and recruitment order in

masseter, and found a positive relationship between spike amplitude and recruitment order. These authors used spike amplitude as an indicator of muscle fibre size, and found that motor units were recruited in order of increasing fibre size. However, as the fast Type II fibres are smaller than the Type I fibres in human masseter, this tends to suggest that the low threshold units must have been composed of the fast fibres, and the larger units composed of the larger slow fibres. This would be the opposite to that which occurs in other human muscles. In interpreting these results, the limitations of using single motor unit spike amplitude as an indicator of muscle fibre size must be taken into account, as the amplitude of a motor unit action potential is highly dependant on the relationship between the recording electrode and the fibres of the motor unit (Buchthal and Schmalbruch, 1980; Miles et al., 1986).

2.6.2.4   Conduction velocity

Motor axon diameter and axonal conduction velocity are correlated with each other (Boyd and Kalu, 1979; Burke et al. 1982), and with motoneuron size (Kernell and Zwaagstra, 1980). Even though the motoneuron is not accessible in humans, its conduction velocity can be measured (Freund et al. 1975). Conduction velocity is an indicator of muscle unit size as larger motoneurons supply a larger number of muscle fibres (Cope and Pinter, 1995). Freund et al. (1975) found that recruitment of motoneurons innervating human hand muscles during voluntary isometric contractions was correlated with axonal conduction velocity. Twitch tension and recruitment

threshold were both found to be correlated with the conduction velocity of motor axons in the upper limb (Dengler et al. 1988).

The recruitment order of motoneurons into the stretch reflex has been shown to be highly negatively correlated with conduction velocity in both homogeneous and heterogeneous muscles of the decerebrate cat (Bawa et al. 1984; Cope and Clark, 1991).

The conduction velocity of a motoneuron can be determined from the H-reflex latency, if the length of the neuronal pathway is known. As this length will be very nearly the same in different motor units of small muscles, comparisons of H-reflex latency can be used as an indicator of relative motoneuron size. As pointed out by Awiszus and Feistner (1993), the measurement of H-reflex latency using an intramuscular electrode includes the time taken for the action potential to reach the electrode within the motor unit. Thus, the latency recorded includes intramuscular transmission time. These authors calculated the intramuscular conduction time from the Macro EMG, and subtracted this from H-reflex latency to determine a corrected latency value (see Section 4.3.2).

2.6.2.5   MacroRep

Stålberg (1980) described an EMG technique for the study of motor units of different sizes, using a modified single fibre EMG electrode. The electrical activity of the shaft of the electrode against a far-away non-muscular site (Macro EMG) was averaged after

being triggered by the firing of the single muscle fibre action potential, in order to extract the contribution of the motor unit to the Macro EMG. The representation of the motor unit in the Macro EMG (MacroRep) is a measure of the cross-sectional area of the muscle units, (the number of muscle fibres in the motor unit multiplied by their cross-sectional area). An intramuscular electrode is preferable when using this procedure: although the unit representation can be produced by averaging the surface EMG with the firing of a motor unit (Schmied et al, 1997), the amplitudes of the reprentations produced from surface electrodes depend on the depth of the unit under investigation and may not be a good indicator of motor unit size (Awiszus and Feistner, 1993).

The amplitude and area of the MacroRep are both positively correlated to the size (diameter) and number of muscle fibres in the motor unit. The area is less affected by electrode position whereas the amplitude is more precise in case of poor baseline measurements. Overall, the peak-to-peak amplitude is the most important parameter of the MacroRep (Stålberg, 1983).

MacroRep area and amplitude are both strongly correlated with the mechanical twitch properties and recruitment thresholds of motor units (Lemon et al. 1990; Vogt et al. 1990). As the number and size of the muscle fibres of a motor unit are good indicators of the size of the motoneuron innervating these muscle fibres (Buchthal and Schmalbruch, 1980; Henneman and Mendell, 1981b), the MacroRep can be considered a good indicator of relative muscle unit and motoneuron size.

The MacroRep has been used in recruitment studies by many authors and has been shown to be related to motor unit size as indicated by twitch tension (Dengler et al. 1989; Vogt et al. 1990) and recruitment threshold (Ashby et al. 1986; Masakado et al. 1994; Jabre and Spellman, 1996).

Thus although the recruitment order of muscle units in masseter has been studied by many authors (eg Goldberg and Derfler, 1977a; Yemm, 1977a; Desmedt and Godaux, 1978a; Desmedt and Godaux, 1979), who have found recruitment to be according to the size principle, there are limitations to the techniques used by these authors to measure muscle unit size. MacroRep is a suitable method for determining muscle unit size in masseter, but has not previously been used when investigating recruitment in masseter.

2.6.3 Effects of synaptic input on recruitment order

Recruitment order is not solely dependent on the size of the motoneurons, there are three neural factors that determine recruitment order; the intrinsic current threshold of the motoneuron (related to its size), the amplitude of the synaptic input that the cell receives and the amount of variability in the motoneuron threshold (Heckman and Binder, 1993). Synaptic input from various sources may change recruitment order, but because of the strong influence of motoneuron size, synaptic input to large motoneurons would need to be ten times stronger than input to small motoneurons, in order to reverse recruitment order (Heckman and Binder, 1990).

The size of the motoneuron will affect the recruitment order due to the higher input resistance of the small motoneurons. Thus a synaptic input onto a small motoneuron will produce a larger excitatory post-synaptic potential (EPSP) than the same input onto a larger motoneuron, due to Ohm's law (V=IR; V = voltage, I = current and R = resistance). For this reason, it is inappropriate to use EPSP size as an indicator of synaptic input to different sized motoneurons, as the different input resistances of the motoneurons obscure the size of the synaptic input (Heckman and Binder, 1990).

If a synaptic input produces the same current in all motoneurons, then it will not influence the recruitment order produced as a result of the intrinsic properties of the cell. If the synaptic input is greatest on high-input-resistant (small) motoneurons, it will increase the range of recruitment thresholds resulting from the intrinsic properties of the cells, and will reinforce the recruitment of motoneurons according to the size principle. If the synaptic input is greatest on low input-resistant (large) motoneurons, then it will compress the range of recruitment thresholds available, and could even change the recruitment order resulting from the intrinsic properties of the motoneurons (Burke, 1981; Kernell and Hultborn, 1990). For example, the input from Deiter's nucleus and the rubrospinal neurons are advantageously distributed to the larger motoneurons, and may have the effect of reversing recruitment order (Heckman and Binder, 1993). The role of inputs that appear to reverse recruitment order may be to increase the "gain" of the whole motoneuron pool input-output function by increasing the participation of motor units with high force capacity during certain activities (Burke, 1981).

2.6.3.1   Input from muscle spindles

Animal studies have shown that Ia input is preferentially distributed to the smaller motoneurons, supporting the size principle (Heckman and Binder, 1988). Ia input from muscle spindles has been found to be distributed to motoneurons according to motoneuron type as well as size, thus Ia input is distributed most onto Type S motor units, and least onto FF motor units for both homonymous (Burke and Rymer, 1976) and heteronymous (Munson et al. 1986) projections. Some caution is needed in the interpretation of these findings, as these studies recorded the EPSP size as an indicator of synaptic input to motoneurons. This may be inappropriate, as the EPSP size is a product of cell resistance and synaptic input, and any deviation in synaptic input may be overshadowed by the effect of motoneuron cell size (Heckman and Binder, 1990). If homonymous Ia input does support the size principle, then the aim of this may be to preserve orderly recruitment in the presence of a wide variety of inputs (Clamann, 1993). This would emphasise the activity of fatigue-resistant, low-force motor units, allowing for precise movements. As the contraction of the S units is slow, Ia input current may also favour slow movements (Heckman and Binder, 1990). This may be a disadvantage when fast movements are needed, as the S units may become overstimulated, although recurrent inhibition of the motoneurons may compensate for this Heckman and Binder (1990) suggested that the synaptic input is different at different force levels, so that a pattern of input preferential to the large motoneurons would be used in fast, strong movements. Similar suggestions of different distribution of

synaptic input were made by Nardone (1990, in humans). A possible mechanism for changing synaptic input at different force levels could be pre-synaptic mechanisms, which are only brought into force at higher force levels. This suggestion is in contrast to the assumption that synaptic inputs remain constant at different force levels (Kernell, 1983).

Mendell et al. (1990) summarised the theories which attempt to explain the greater effectiveness of Ia inputs onto small motoneurons. Whereas Henneman (1965) suggested that the density of boutons was the same on small and large motoneurons, this would result in a greater number of boutons on large motoneurons. Burke (1981) suggested that the number of boutons was the same on large and small motoneurons. Luscher et al. (1980) stated that the anatomical density on large and small motoneurons was the same, but that there were many inactive terminals on large motoneurons, whereas Collins et al. (1984) concluded that the synapses on smaller motoneurons were stronger, in that they released more transmitter. Whatever the mechanism, it seems clear that in animal soleus, EPSPs resulting from Ia input support the size principle.

Input from Ia afferents has been shown to support the size principle in human wrist extensors. Schmied et al. (1997) showed that the probability of a response of a motor unit to Ia input, induced by tendon taps, was related to the motor unit's functional characteristics. Motor units with low recruitment thresholds, long contraction times, small twitch tension and small Macro EMG potentials had the largest reflex responses, with the longest latencies. Smaller H-reflex responses have also been found in small

motoneurons in human soleus, where motoneuron size was estimated from the H-reflex latency (Awiszus and Feistner, 1993). However, the same caution applies to the interpretation of these results as to the findings of the animal studies; that EPSP size may not necessarily represent synaptic input as it is affected by motoneuron input resistance.

Buller et al. (1980) studied changes in the probability of firing of motor units active during voluntary muscle contraction in the first and second dorsal interosseous of the hand in humans, in response to muscle afferent stimulation. Motor units recruited at low levels of voluntary contraction strength were more responsive to muscle afferent input than units recruited at high contraction strengths, and the authors concluded that the excitatory effect of muscle afferent input is weighted in much the same way as the input producing voluntary contraction. When comparing H-reflex responses in large and small motor units, as indicated by the size of the motor unit representation in the Macro EMG, synaptic efficacy to small and large units in human tibialis anterior has been shown to be the same (Ashby et al. 1986).

In contrast, two studies have suggested that Ia afferent input was more effective on larger motoneurons. Preferential input from Ia afferents onto large motoneurons was suggested by larger H-reflexes in high-threshold motor units in human tibialis anterior (Semmler and Türker, 1994). Mazzocchio et al. (1995) used a technique where magnetic brain stimulation was used to clear the axons of large motoneurons from antidromic impulses. H-reflexes were found to be larger in the larger muscle units, with

the conclusion drawn that Ia input was preferentially skewed in favour of large motoneurons in the human abductor digiti minimi.

In the human soleus muscle, recruitment by electrical stimulation to the posterior tibial nerve produced a different recruitment order from that produced by voluntary contraction of the muscle (Davies et al. 1993), suggesting that Ia input was not distributed according to the size principle. However, Calancie and Bawa (1985) found that motor units were recruited in the same order during the stretch reflex as during voluntary contraction. Differences between electrical and mechanical stimulation may have accounted for these differences. Romaiguere et al (1993) found that the order of recruitment in the wrist extensors was the same during voluntary contractions and the tonic vibration reflex. However, limitations in the methods of motor unit recognition in this study place constraints on interpretation of the results.

Thus in human work there are conflicting findings about the effective distribution of Ia input onto homonymous motoneurons. This may reflect arrangements in different muscles, or different methodologies used. In animal preparations, synaptic activity may have been affected by anaesthesia or decerebration. In methodologies utilising the H-reflex, the electrical stimulation used may have resulted in the stimulation of afferents other than those from the muscle spindles, in particular the Ib afferents from the golgi tendon organs. However, as the Ib fibres transmit at a slower velocity than the Ia fibres, this should not affect the first part of the composite EPSP. Also, the presence or otherwise of golgi tendon organs in adult human masseter is unclear. A variety of

different muscles have been used in previous studies. A different organisation in different muscles may explain the different results.

Muscles are usually voluntarily activated during experiments using the H-reflex or stretch reflex to investigate Ia distribution. The changes in firing pattern of these motor units in response to the stimulus is recorded using peristimulus time histograms (PSTH). In some instances, motor units that are not voluntarily recruited will be brought into action by the stimulus. These units can be termed "stimulus-recruited" units, as they fire only directly in relation to the stimulus. Stimulus-recruited units have been recorded in response to stretch stimulus of the jaw muscles (Miles et al. 1995). These were large units compared to those that were tonically active, and they fired only in the long-latency response. The presence of stimulus-recruited units may also indicate that the Ia input is distributed to units in a different order than would occur if distribution were according to the size principle.

2.6.3.2   Input from cutaneous receptors

Input from cutaneous receptors has been shown to change recruitment order. Cutaneous stimulation of the human finger results in an increase in recruitment threshold of low threshold units and a decrease in recruitment threshold of high threshold units (Garnett and Stephens, 1981) or a decreased recruitment threshold in high threshold motor units without affecting the recruitment threshold of low threshold motor units (Kanda and Desmedt, 1983). In animals, cutaneous input results in polysynaptic inhibition to Type S

motoneurons and excitation of fast motoneurons (Burke et al. 1973). Kanda et al. (1977) found that electrical or natural stimulation of the sural nerve suppressed activity in low-threshold motoneurons in the decerebrate cat medial gastrocnemius, while activating high-threshold motoneurons. The functional implications of the effect of cutaneous input is that less descending drive is needed to produce a given force.

Due to the effect of cutaneous stimulation on motoneuron firing, care must be taken to take this into account during experiments designed to determine motoneuron responses to Ia input.

2.6.4 Recruitment in jaw muscles

Recruitment order in the jaw muscles has been studied by many authors, who have generally agreed that recruitment occurs according to the size principle (Goldberg and Derfler, 1977a; Yemm, 1977a; Clark et al. 1978; Desmedt and Godaux, 1979; Lund et al. 1979). These authors have used spike-triggered averaging of force, spike amplitude and force recruitment threshold as indicators of muscle unit size. As discussed previously, there are serious limitations in these methodologies when used to determine muscle unit and motoneuron size in the jaw muscles.

The lowest sustainable firing frequency that can be maintained by a motor unit depends on the intra-oral task being performed, indicating that descending neural drive to masseter is task dependant (McMillan and Hannam, 1992). Masseter motor units are

activated selectively during specific intraoral tasks, suggesting that different populations are activated within the motoneuron pool. This may produce recruitment of motor units contrary to the size principle in response to different distribution of inputs for different tasks (McMillan and Hannam, 1992). Thus when investigating motor unit recruitment in masseter it is necessary to ensure consistency in the biting task investigated.

The MacroRep has been shown to be a good indicator of muscle unit size in the muscles of the limbs, providing a measure of the cross-sectional area of the muscle unit (number of fibres multiplied by the cross-sectional area of each fibre). This should therefore provide a useful tool for establishing motor unit recruitment in masseter. However, one note of caution needs to be considered. In the limb muscles, small motoneurons supply small motor units, which have fewer fibres and are composed of small Type I fibres (Buchthal and Schmalbruch, 1980). Correspondingly, large motoneurons supply large motor units with many larger fibres. Thus there should be a wide range of MacroRep sizes, and the MacroRep size can be used as an indicator of motor unit and motoneuron size. However, as described previously, Type II fibres in masseter are actually smaller in diameter (about half the size) than the Type I fibres (Eriksson, 1982). Thus, the range of MacroRep amplitudes might be expected to be compressed. As the Type II fibres are of a smaller diameter than the Type I fibres, it may even be that the MacroRep of the motor units containing Type II fibres is smaller than that containing Type I fibres (Figure 2). An important issue in determining whether MacroRep will reflect motoneuron size is the number of muscle fibres in the masseter motor units. If there is a very wide discrepancy in the number of muscle fibres in the large and small motor units, then this

Limb Muscles

Motoneuron			
Muscle unit			
Muscle fibre type	Type I	Type IIA	Type IIB
Cross-sectional area			
MacroRep			

Masseter

Motoneuron		
Muscle unit		
Muscle fibre type	Type I	Type IIB
Cross-sectional area	?	?
MacroRep	?	?
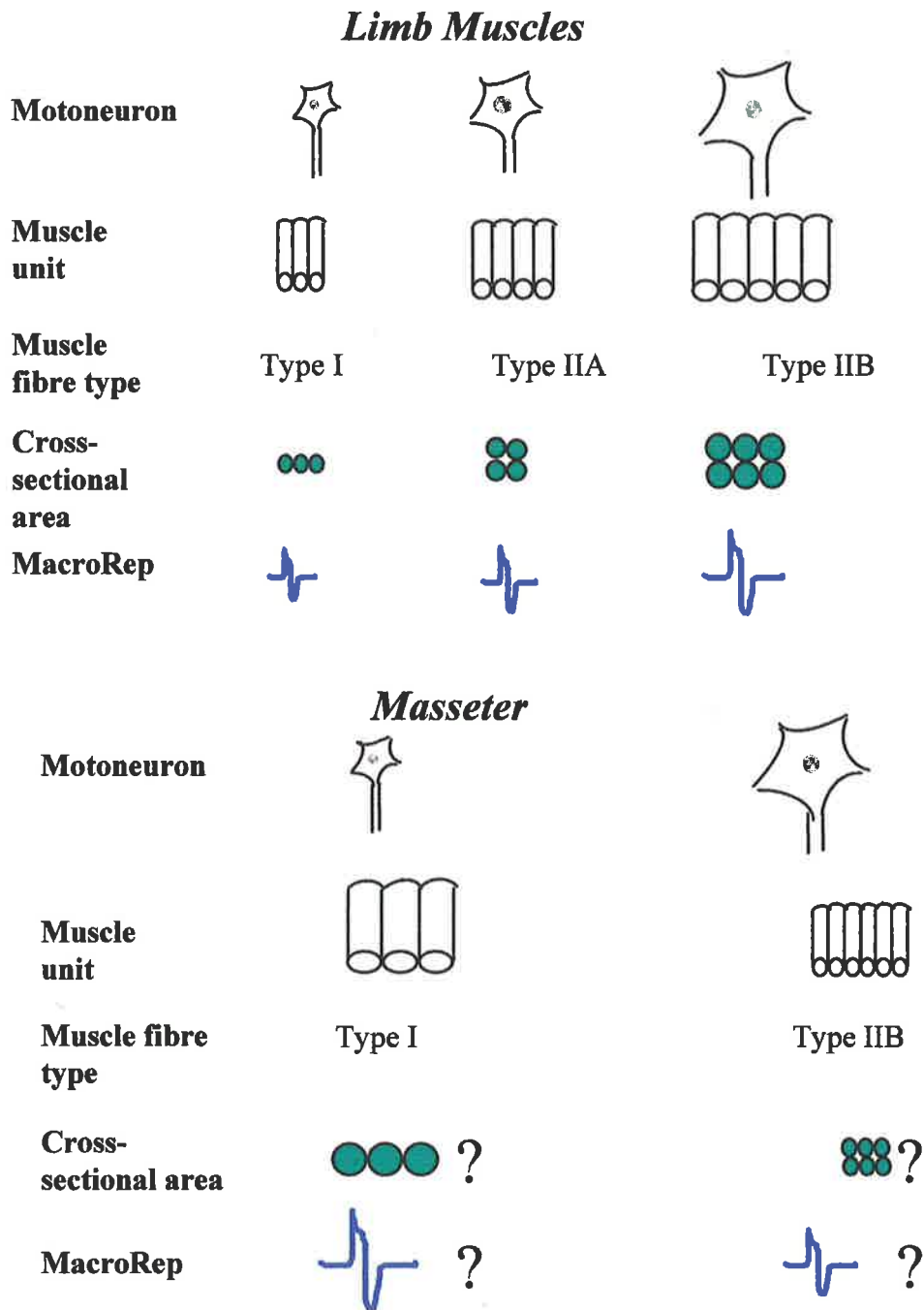
Figure 2. Relationship between Motoneuron size and MacroRep in Limb and Masseter Muscles.

In the limb muscles, small motoneurons supply muscle units composed of a small number of small Type I fibres. Thus small motoneurons have a small MacroRep. Due to the small size of the Type II fibres in masseter, the MacroRep could possibly be larger in muscle units supplied by small motoneurons.

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will compensate for the smallness of the Type II fibres. As discussed previously (Section 2.3) it is likely that there is a large range in the number of fibres in masseter motor units. Whether this is sufficient to compensate for the small size of the Type IIB fibres is at present unknown.

Thus, although MacroRep size reflects *muscle unit* size, it may not reflect *motoneuron* size in masseter. For MacroRep to be used as an indicator of motoneuron size, and to be used in recruitment studies, the relationship between MacroRep and motoneuron size needs to be established.

Chapter 3. Stretch Reflex and H-reflex

Stretch reflexes and H-reflexes result from the application of either a stretch stimulus, usually in the form of a tap over a muscle tendon, or an electrical stimulus applied to the nerve supplying a muscle. Both the stretch reflex and the H-reflex are recorded in the muscle of interest as an electrical signal, using electromyography. Stretch and H-reflexes are generally used to examine the excitability of the motoneurons and the effects of various synaptic inputs (Fujii, 1979; Schieppati, 1987). The H-reflex consists of a short-latency excitation, whereas the stretch reflex consists of a short-latency component and a longer latency component, which is probably due to a second pathway from the muscle spindles traversing the motor cortex to activate the motoneurons by corticospinal pathways (Poliakov and Miles, 1994).

The short-latency components of both the stretch reflex and H-reflex are often considered to be monosynaptic (Munro and Griffin, 1971). However, oligosynaptic as well as monosynaptic transmission from group Ia afferents to homonymous motoneurons has been demonstrated (Jankowska, 1984). Burke et al. (1989a) argued that the stretch reflex is unlikely to be purely monosynaptic as the rising phase of the composite excitatory post-synaptic potential (EPSP) lasts up to 10 ms, and much of this is spent raising the motoneuron membrane potential to threshold. Motoneurons discharge in the last half of the EPSP, some milliseconds after the onset of monosynaptic excitation. This makes it likely that only the early part of the tendon jerk is

monosynaptic. The last phase of electrically-induced EPSPs may be affected by Ib inhibitory effects, suppressing the last-discharging motoneurons of the reflex response (Buller et al. 1980; Schieppati, 1987), as Ib inhibitory effects can start 0.8 ms after the onset of Ia excitation (Pierrot-Deseilligny et al. 1981). However, the presence of golgi tendon organs in human masseter has not been clearly established, so their effect on masseter stretch reflexes cannot be determined. Additionally, the selectively of the tendon tap to stimulation is very good if small vibrations are used (less than 1mm) (Roll et al, 1989). The EPSP rise time in the H-reflex is very short, probably less than 2 ms, so it is less likely that it is subject to any significant post-synaptic temporal summation (Schieppati, 1987), and is more likely to be effectively monosynaptic (Burke, 1989a).

The stretch reflex and the H-reflex have often been considered to have the same central connections, the only difference between them being the presence of the muscle spindles in the stretch reflex circuit (van Boxtel, 1986). Based on this assumption, the H-reflex and stretch reflex have been compared to allow an assessment of spindle sensitivity (Schieppati, 1987; Burke, 1989a). However, there are many differences between the behaviour of the stretch reflex and the H-reflex, suggesting that the H- and stretch reflexes differ in fundamental ways. For example, the H-reflex demonstrates greater amplitude reduction in response to repeated stimulation and also shows greater inhibition from vibration (van Boxtel, 1986). The stretch reflex is also easier to elicit and is more widespread that the H-reflex (Burke, 1989a).

The differences in the stimuli applied to elicit stretch and H-reflexes may be partly responsible for the different behaviours of the reflexes. The H-reflex is elicited by electrical stimulation of the muscle nerve whereas the stimulus in a stretch reflex usually takes the form of a tap. The electrical stimulus is likely to excite the Ib afferents and cutaneous afferents as well as the muscle spindle afferents. The electrical stimulus may excite different Ia afferents than the tap stimulus, as it will preferentially excite the largest Ia afferents first (Wiederholt, 1970). The afferent volley produced by a tap stimulus will not contain as much Ib activity, but the stimulus is less selective. The stimulus from a tap results not so much from the tap, as from the vibration wave it establishes, which propagates away from the stimulus, exciting mechanoreceptors in skin and muscle (Burke, 1989a). Therefore a tap may result in stimulation of the antagonists and synergists as well as the muscle of interest.

3.1   Characteristics of H-reflex responses

3.1.1 Extinction

Electrical stimulation of a mixed nerve (like the masseteric nerve) can result in several responses, including H-reflexes, M-waves and F-responses, which may be recorded in the muscle. When the aim of the study is to investigate H-reflexes, the stimulus durations usually used are 0.5 - 1.0 ms, as these result in preferential stimulation of the afferent fibres (Hugon, 1973). Thus the H-reflex is recorded as a result of the excitation of the motoneurons resulting from an excitatory afferent volley. As stimulation intensity is increased the H-reflex amplitude initially increases, then decreases, and becomes

extinct with further increases in stimulus intensity. As stimulation intensity is increased, direct stimulation of the motor axons occurs, resulting in the production of an M-wave. The M-wave amplitude increases with increasing stimulus intensity, until a plateau is reached. The relationship between the sizes of the H-reflex and the M-wave and stimulus intensity has been described in the lower limbs, as shown in Figure 3. To quantify H-reflex size, it is expressed as a ratio of M-wave amplitude (Hmax/Mmax), although the advantages of using other methods, such as the ratio of the intensity-response curves of the H-reflex and M-waves and threshold stimulus intensity values for both reflexes have also been discussed (Funase et al. 1996). De Laat and Macaluso (1995) investigated H-reflexes and M-waves in human masseter using the Hmax to Mmax ratio, and found that these values were much lower in masseter (H-reflex amplitude about 13% of M-wave) than in soleus (50 - 70%).
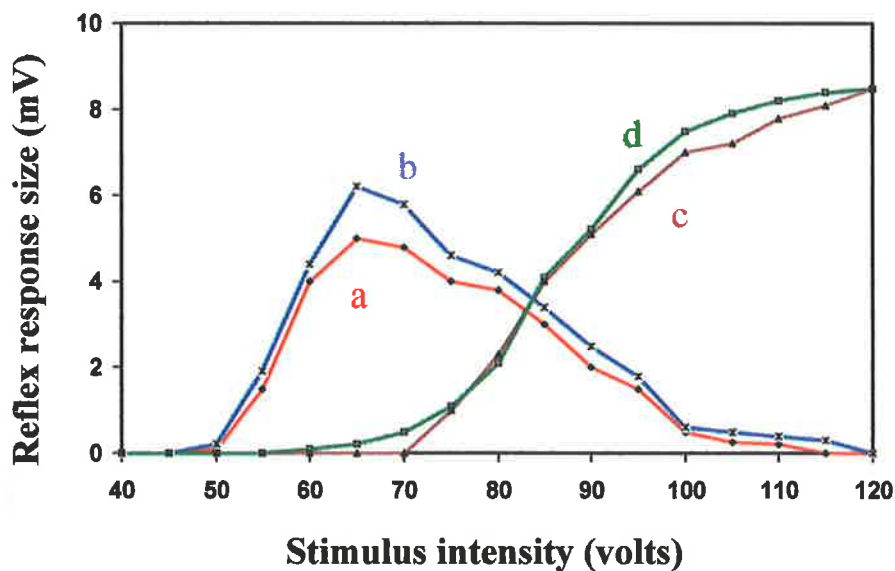
Figure 3. Recruitment curves of the soleus muscle.

(a)   H-reflex with soleus muscle relaxed. (b) H-reflex elicited during voluntary contraction of soleus. (c) M-wave with soleus muscle relaxed. (d) M-wave during contraction of soleus. At low stimulus intensities, H-reflexes are produced with no M-wave. As stimulus intensity is increased, M-waves are produced. As stimulus intensity is increased further, H-reflex amplitude decreases while the M-wave continues to increase. H-reflex, but not M-wave amplitude, is increased when the muscle is contracting. Redrawn from Schieppati (1987).

The extinction of the H-reflex with higher stimulus intensities is usually thought to be due either to an antidromic volley in the motor fibres colliding with the reflex action potentials, or to a refractoriness in the motoneuron pool, as a result of the antidromic volley (Gottlieb and Agarwal, 1976). If this is the mechanism of H-reflex extinction, then the H-reflex transmission in the largest motor axons or motoneurons would be affected first, as the thick fibres would be affected at the lowest stimulus intensity. However, Hilgevoord et al. (1995) found that the mean decrease in H-reflex amplitude at higher stimulus intensities, relative to the maximum amplitude, remained the same when the H-reflex was depressed by tendon vibration. This result is hard to reconcile with an explanation of extinction based on an ordered occlusion. The authors suggested that H-reflex extinction was therefore due to input from Ib afferents from Golgi Tendon Organs (GTOs) or to Renshaw inhibition. Whether Renshaw inhibition is present in the masticatory system is debatable. No evidence of axon collaterals has been found in masticatory motoneurons in the adult cat, suggesting that recurrent collateral inhibition is not available in the masticatory system (Shigenaga et al. 1988). However, injection of horseradish peroxidase into the somata of jaw-elevator motoneurons showed the presence of axon collaterals in rats (Moore and Appenteng, 1989) suggesting that these motoneurons may exert recurrent synaptic effects. The presence or otherwise of GTOs in human masseter has not been confirmed. GTOs have been identified in masseter attachments in the kitten (Lund et al. 1978) but they have not been demonstrated in jaw-closing muscles in humans (Lucas et al. 1984). De Laat and Macaluso (1995) considered that a direct effect of the antidromic motoneuronal volley on the

refractoriness of the motoneuron was the most likely explanation for extinction. The collision theory was considered unlikely as the cause, as the short distances in the masseteric nerve made it unlikely that the time for transmission along the Ia afferent fibre, plus a synapse on the motoneuron, would equal antidromic conduction time.

3.1.2 Differentiation from F-waves

The F-wave is caused by back-firing of a small proportion of the motoneuron pool (Trontelj, 1973) in response to antidromic excitation, when supramaximal stimulation is applied to a motor nerve (Dengler et al. 1992). H-reflexes behave in characteristic ways, enabling them to be distinguished from F-waves, which occur at a similar latency. Whereas H-reflexes occur at lower intensities that M-waves, F responses have a higher threshold than the M-wave. H-reflexes occur more easily in the presence of voluntary contraction, as the general excitability of the motoneuron pool is increased, whereas F responses are unaffected by voluntary contraction (Fujii, 1977). F-waves are also not susceptible to extinction with increased stimulus intensity.

3.1.3 Low frequency depression

The amplitude of the H-reflex is depressed if the stimulation rate is increased above 0.1 - 0.5Hz (Cook, 1968; van Boxtel, 1986). This low frequency depression probably occurs because of the partial depletion of the available transmitter store (Capek and Esplin, 1977). Small H-reflexes are more susceptible to low-frequency depression than large H-reflexes (Burke et al. 1989b; Floeter and Kohn, 1997). The H-reflex is more susceptible

to low frequency depression than the stretch reflex, perhaps because of the greater contribution of oligosynaptic circuits to the stretch reflex (van Boxtel, 1986). Voluntary contraction in a muscle can overcome the effects of low frequency depression (Burke et al. 1989b), so in experiments investigating H-reflex the stimulation frequency should be kept low or a voluntary contraction should be maintained in the muscle tested (van Boxtel, 1986). Burke et al. (1989b) also found that H-reflexes can be produced at lower stimulus intensities during voluntary contractions, and that a more clear separation of the M-wave and H-reflex then became possible. Additionally, variability in recordings of H-reflex onset latency are reduced in the presence of a voluntary contraction. This has been explained by Burke et al. (1989b): when the muscle is relaxed much of the EPSP is dissipated in raising the excitability of the motoneuron pool to threshold (Burke et al. 1984). Therefore, differences in the ability of subjects to relax can change the reflex latency. If motoneuron excitability is stabilised by the presence of a voluntary contraction, then this latency variability will be reduced.

3.1.4 Effects of motor unit firing frequency

There are controversial findings concerning the influence of motoneuron prestimulus firing frequency on the size of the H-reflex response. Several authors have shown that the stretch reflex size (Ashby and Zilm, 1982a; Brouwer et al. 1989; Miles et al. 1995) and the H-reflex size (Ashby and Zilm, 1982a; Miles et al. 1989; Awiszus and Feistner, 1995; Schmied et al. 1997), are independent of firing frequency.

Contrary results have been found by Jones and Bawa (1995), who examined the effects of synchronous Ia volleys on the firing probability of repetitively firing human motoneurons, firing at fast and slow rates. When the stimuli were given at random with respect to the times of the motor unit spikes, the magnitude of the PSTH response (response probability) was significantly lower at a faster firing rate. If the stimuli were given at various known times during the interspike interval, the response probability was increased as the stimuli were given progressively later during the interspike interval. The response probability was greater at a higher firing rate. These results conflict with those of other authors, the most likely reason being because the response probability in this study was not normalised to take into account the number of stimuli received by rapidly and slowly firing motoneurons.

Thus, it seems unlikely that firing frequency will affect H-reflex response size. However, when comparing H-reflexes in different motor units, it is appropriate to record motor unit firing frequency so that it can be established whether differences in firing frequency could be a possible explanation for changes in H-reflex response.

3.1.5 Effects of vibration

When vibration is applied to a limb muscle, the stretch and H-reflexes are depressed (de Gail et al. 1966). This depression of the stretch and H-reflexes, occurring at the same time as the development of a tonic contraction, is known as the vibration paradox (de Gail et al. 1966; Lance et al. 1973). In the cat, the inhibition of reflexes is accompanied

by primary afferent depolarisation and a dorsal root potential. As the reflex inhibition and the dorsal root potential can be abolished by picrotoxin (Rudomin, 1990), and the excitability of the motoneurons is unchanged (Schmidt, 1971), Gilles et al. (1969) and Barnes and Pompiano (1970) suggested that the reduction in monosynaptic reflexes occurred due to pre-synaptic inhibition of the Ia afferent input (in the cat). In humans, inhibition of monosynaptic reflexes can occur through the vibration of a muscle or its synergists. This has been demonstrated in the soleus (Dindar and Verrier, 1975) and the flexor muscles of the forearm (Hendrie and Lee, 1978).

Although pre-synaptic inhibition of Ia afferent input is generally accepted as the mechanism by which H- and stretch reflexes are reduced in response to vibration, an alternative explanation could be that the Ia afferents are occluded by the vibratory stimulus. This is not likely, as the inhibition can occur with vibration frequencies as low as 40Hz (Ashby et al. 1987). The depression can also not be explained by post-synaptic inhibition of the motoneuron as there is no change in the firing rate of the motoneuron, and there is no change in the effectiveness of the post-synaptic potentials produced by stimulating cutaneous afferents (Ashby et al. 1987). Abbruzzese et al. (1997) showed that muscle vibration does not affect the post-synaptic excitability of the motoneuron pool, and that the depression of the H-reflex results from a pre-synaptic block of the Ia spindle afferents. The responses to vibration could be due to changes in the electrical threshold of the muscle afferents to vibration, although no evidence for such changes has been found (Abbruzzese et al. 1985; Ashby et al. 1987).

Although vibration stimulates the primary and secondary spindle endings, and the golgi tendon organs, it is the primary endings that are driven at the highest frequencies (Burke et al. 1976). There is no increase in the vibratory inhibition when static stretch is applied to the muscle, suggesting a limited influence of PSI on the secondary afferents (Dindar and Verrier, 1975). The GTOs are not likely to be involved in the depression of the reflexes, as voluntary muscle contraction, which preferentially stimulates the golgi tendon organs, does not replicate the inhibition (Burke, 1989a).

Although vibration depresses H-reflexes in the limb muscles, the opposite occurs in masseter, where stretch and H-reflexes have been found to be potentiated by vibration (Godaux and Desmedt, 1975), although no other reports of this phenomenon have been described. On the basis of the facilitation of H- and stretch reflexes by vibration, Desmedt and Godaux (1980) suggested that pre-synaptic inhibition may not be present on the Ia afferents of the jaw-closing muscles (Desmedt and Godaux, 1978b) (see Section 3.4.2).

3.2   H-reflexes in masseter

H-reflexes in masseter have been studied by many authors, using a variety of stimulation and recording techniques, as summarised in Table 1. The latency of the masseteric H-reflex is about 6 ms. This latency is concomitant with a monosynaptic reflex: the length of the masseteric nerve has been measured as 11 cm (Fujii, 1977) or 8.1 cm (Munro and Griffin, 1971). If the conduction velocity of the masseteric nerve fibres is about 69

Authors	Stimulation Method	Recording	Number of Subjects	M-wave latency (onset)	H-reflex latency (onset)	H reflex size (% M wave)
Fujii and Mitani 1973	surface	surface EMG	5	about 2ms (peak)	about 6ms	nr
Mitani, 1974	surface	surface	nr	about 2ms	about 6ms	nr
Godaux and Desmedt 1975	bipolar needle, mandibular notch	surface EMG	17	1.3 - 1.9ms	4.6 - 5.8ms	11 - 37 (mean 24%)
Cruccu and Bowsher, 1976	intracranial/ foramen ovale	surface/ needle EMG	16	2.2ms	5.4 - 5.7 ms (peak)	15 - 50
Fujii 1977	Surface, mandibular notch	surface EMG	21	about 2ms	about 6ms	nr
Cruccu, 1989a	bipolar needle, mandibular notch	surface EMG	50	1.6 +/- 0.2ms	5.8 +/- 0.7ms	about 25
Cruccu et al 1989b	bipolar needle, mandibular notch	surface/ needle EMG	25	1.56 +/- 0.3ms	6.1 +/- 0.9ms	about 25
de Laat and Macaluso 1995	monopolar needle, mandibular notch	surface EMG	10	1.4 - 2.1ms	5.6 - 6.7ms	13
Macaluso and de Laat, 1995a	monopolar needle, mandibular notch	surface EMG	24	nr	5.76 - 6.21ms	2 - 32 (mean 11.7)

Table 1. H-reflexes in Human Masseter

Summary of previous work investigating H-reflexes in human masseter. Details of stimulation and recording methods, subjects and latency of M-waves and H-reflexes are included. The size of the H-reflex is expressed as a percentage of the size of the maximum M-wave. nr = not reported.

metres per second (Gasser and Grundfest, 1939) then both afferent and efferent conduction times will be about 1.6 ms (or 1.8 ms if the conduction velocity of 58m/s calculated by Cruccu et al. (1986) is used). Cruccu and Bowsher (1986) calculated that the central delay was 1.8 ms. Allowing 0.7 ms for end-plate delay (Fujii, 1977) suggests an H-reflex latency of about 6.1 ms.

H-reflex recruitment curves in masseter have been produced by several authors, and are similar to those demonstrated in the lower limbs (Godaux and Desmedt, 1975; de Laat et al, 1998). However, when compared to maximum M-wave amplitude, H-reflex amplitude is small in masseter, especially in light of the fact that they are elicited in the presence of voluntary contractions, which usually increases H-reflex amplitude (de Laat and Macaluso, 1995; Macaluso and de Laat, 1995a).

H-reflexes are small and can be difficult to elicit in masseter (Fujii and Mitani, 1973; Macaluso and de Laat, 1995a), and in the muscles of the hand (Schieppati, 1987; Mazzocchio et al. 1995). Mazzocchio et al. (1995) describe several reasons why there may be difficulty in eliciting H-reflexes in the hand muscles, as described below, and these reasons may be applicable to the jaw muscles as well.

1. If motor axons have a lower threshold to electrical stimuli than the Ia fibres, then H-reflex discharges elicited by sensory inputs would be prevented from reaching muscles and being recorded, by collision with antidromic impulses. This suggestion is supported by the finding that the motor fibres in the masseteric nerve are thicker than

the spindle primary afferents in the cat (Morimoto et al. 1982). If this is the case in humans, then H-reflexes will be difficult to elicit, as the thicker motor fibres will be stimulated at low threshold by the electrical stimulus and the H-reflex response will be blocked by the antidromic activation of the motor fibres.

2. Weak monosynaptic projections from Ia afferents onto the motoneurons will make H-reflexes small and difficult to record. In rats, the Ia afferents from the masseteric nerve have been found to project to only about 10% of the motoneurons (Appenteng et al. 1978). If applicable to humans, this would account for both the difficulty of eliciting H-reflexes and their small amplitude in comparison to M waves (Goldberg and Derfler, 1977a; Macaluso and de Laat, 1995a). The EPSP may be insufficient to depolarise the motoneuron to a firing level when the muscle is resting, but in the presence of a voluntary contraction, the excitability of the motoneuron pool is increased (Garnett and Stephens, 1981), and an H-reflex may then occur. This occurs in masseter, where voluntary contractions of up to 40% of maximum are necessary in order to elicit H-reflexes (Macaluso and de Laat, 1995a).

3. There may be a skewed distribution of Ia effects in the motoneuron pool, with excitation being prevalent among the high-threshold units. In this case, H-reflexes would not be revealed when the muscle was in the relaxed state or at a low level of contraction. This pattern of Ia distribution has been demonstrated in the hand muscles. Mazzocchio et al. (1995) used magnetic brain stimulation to provide a volley to collide with an antidromic volley in the large motor axons, thus clearing

these axons to contribute to an H-reflex. These authors concluded that H-reflexes are difficult to obtain in the hand muscles because the Ia input is ineffective on small, low threshold motoneurons. Several reasons for the increased effectiveness of Ia input on large motoneurons were discussed. It was thought unlikely that Ia terminals were preferentially distributed to large motoneurons, as this would contradict the normal findings in the limbs. Thus, either the input to the small motoneurons may be pre-synaptically inhibited in some way, or a concurrent inhibitory process could mask the distribution of Ia inputs. A preferential input from Ia afferents onto large motoneurons could provide a synaptic organisation favouring motoneurons with fast axons. This would provide increased accessibility of the fast motor units of the hand to cortical control, needed during precision activities of the hand (Mazzocchio et al. 1995).

3.3 Stretch reflexes in Masseter

Stretch reflexes in the limb muscles consist of short-latency and long-latency excitatory components, with most force changes in the muscle being associated with the long-latency component (Miles and Poliakov, 1997). Masseter also demonstrates long-latency and short-latency components of the stretch reflex (Poliakov and Miles, 1994) although it had previously been thought that only the short-latency component was present (Cooker et al. 1980). Short-latency responses were shown to have a latency of 10-20 ms, and long-latency responses about 35 ms, lasting until about 100 ms.

Miles et al. (1995) investigated stretch reflex responses in motor units in human masseter and found that on occasion, non tonically-active motor units responded in the long-latency component of the stretch reflex. This was attributed to the shape of the compound EPSP produced by the stimulus, with the long-latency component being larger. Of the tonically active motor units, 35% showed no response to stretch. No relationship was demonstrated between recruitment threshold of the motor units and their responsiveness to stretch. However, as acknowledged out by the authors, most of the units in the study were low-threshold, so such a relationship may have been present but not demonstrated.

The input from the muscle spindles, resulting in the masseter stretch reflex, has been shown to be important in stabilising the position of the mandible during movements of the body (Lund and Olsson, 1983b). For example, during locomotion, the vertical movements of the head would result in an oscillation of the mandible without the stretch reflex activity evoked in the jaw-closing muscles (Larson et al. 1981; Lund, 1990). Lund et al. (1984) showed that the level of jaw-closer EMG activity varied with the up-and-down movement of the head (in cats) and was strongly correlated with movement in the vertical plane. It should be noted that the stretch reflex in the jaw-closing muscles is almost certainly not exclusively responsible for maintenance of jaw posture. For example, the vestibular receptors (Funakoshi and Amano, 1973) and neck mechanoreceptors (Griffiths et al. 1983) also have inputs onto jaw-closing motoneurons.

Input from muscle spindles in the jaw-closing muscles may also play a part in the control of jaw movements during chewing and swallowing of food (Miles et al. 1995). Although the basic movements of chewing can be produced within the central nervous system by the central pattern generator located in the brain stem (Nozaki et al. 1986), input from peripheral receptors is required to modify the pattern in response to changing food position and consistency (Luschei and Goldberg, 1981; Ottenhoff et al. 1992; van der Bilt et al. 1995). Although there is little activity in the jaw-closing muscles during up-and-down jaw movements, the activity increases greatly when resistance is encountered due to the presence of food in the mouth (van der Glas et al. 1987; Ottenhoff et al. 1992). Input from peripheral receptors will also be necessary for the performance of non-rhythmical movements such as speech, prey capture and incisal breaking (Dessem, 1995). Dessem (1995) found that the muscle spindle afferents could be activated by tooth displacement forces of less than 0.2N, suggesting that the muscle spindle afferents could contribute to the modulation of chewing.

The role of the input from the muscle spindles during chewing is controversial. In monkeys, cutting the afferent supply from the muscle spindles does not affect chewing patterns or muscle activity, although a preference for chewing on the unaffected side develops (Goodwin and Luschei, 1973). Destroying the trigeminal mesencephalic nucleus (Mes V), as occurred in that study, would also have affected some of the periodontal mechanoreceptor afferents, which may have been responsible for the change in chewing side.

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Other authors (Morimoto et al. 1989a; Morimoto and Nagashima, 1989b) have concluded that the muscle spindles do contribute to the control of jaw-closing muscle activity during chewing. In experiments on anaesthetised rabbits, when the input from muscle afferents was removed by a lesion of the trigeminal mesencephalic nucleus (Mes V), where the cell bodies of the primary afferents are contained, the facilitatory effects of chewing on a test strip were reduced to 75-80% of control values. Kainic acid was injected along the MesV one week before the experiment, to ensure that the changes were not due to inadvertent destruction of neurons surrounding the MesV. When the maxillary and inferior alveolar nerves (containing the afferents from the periodontal mechanoreceptors, facial skin, gums and mucous membranes) were also sectioned, the facilitation of the masseter activity by chewing on a test strip was also completely eliminated. Thus, the muscle spindles may contribute to the development of jaw-closing force during chewing. Of interest is the finding that the masseteric response to facilitation of chewing by a test strip inserted between the teeth was stronger in the anterior part of the masseter, where the most muscle spindles are located (Morimoto et al. 1989a). If closing of the jaw is stopped by a tough piece of food, spindle afferent input would increase, supported by discharge of the intrafusal muscle fibres. This would increase alpha motoneuron activity through the stretch reflex arc (Morimoto and Nagashima, 1989b). It is likely that the excitation of masseter motoneurons in this case is due to the primary afferents, as the afferent discharges of the secondary afferents do not increase with an increase in extrafusal fibre activity (Appenteng et al. 1978). Dessem (1995) considered that the muscle spindles would only contribute to the development of biting force when the level of fusimotor activity ("fusimotor set":

Prochazka, 1989) was high, such as in strong biting or aggressive/defensive behaviours. In these situations, the muscle spindle afferents would provide a short-latency excitatory pathway to the jaw-closing motoneurons.

Ottenhoff et al. (1992) considered that the muscle spindles could not be the receptors responsible for the development of the additional muscle activity (AMA) required to overcome the resistance of food during chewing, as the latency of the stretch reflex was too short to correspond with the AMA latency of 22 - 24 ms. They stated that the long-latency component of the stretch reflex could account for this latency, but that the stretch-reflex in the masseter did not demonstrate a long-latency component. However, long-latency components of the stretch reflex have been demonstrated in masseter (Poliakov and Miles, 1994), and these are associated with most force changes (Miles and Poliakov, 1997).

Further light may be cast upon the role of the muscle spindles in masseter if the effectiveness of the Ia input onto the jaw muscle motoneurons is known. The usual pattern of distribution of Ia input onto small motoneurons supports the role of the muscle spindles in the control of posture. However, as chewing and speaking are activities involving fast movements of the jaw, more effective input of the Ia afferents onto the larger motoneurons may serve this type of activity better.

3.4 Modulation of reflexes

Modulation of the stretch reflex in the jaw-closing muscles is necessary to enable chewing to occur (Lund and Olsson, 1983b). For example, when the jaw is opening the jaw-closing muscles will be stretched. In this situation, excitation of the jaw-closing motoneurons is undesirable, so the stretch reflex needs to be modulated.

Van der Bilt et al. (1997) investigated modulation of the stretch reflex in the jaw-closing muscles during dynamic movements of the jaws. Resistance from food was simulated by the addition of external forces to the mandible, resisting closing of the jaw. During jaw-opening, the stretch reflex was very weak or non-existent. The stretch reflexes were strong at the onset of jaw-closing, during closing and at occlusion.

Reflex responses to stimuli may be modulated by pre-synaptic or post-synaptic influences. For post-synaptic modulation, modulating influences are applied directly to the motoneuron, increasing or decreasing its excitability. For pre-synaptic modulation, the transmission of impulses from the afferent fibres to the motoneuron is modulated.

3.4.1 Post-synaptic inhibition

The amplitude of the stretch or H-reflex reflects the excitability of the motoneuron pool, which results from the net total of supraspinal or segmental excitatory and inhibitory influences (Capaday and Stein, 1989). Lobbezoo et al. (1993b) and Lund (1983a) have

demonstrated that when the EMG activity of the jaw-closing muscles is higher, such as during isometric clenching, the reflex responses are greater.

Lund (1990) suggested that post-synaptic factors were most important in modulating the stretch reflex in the jaw-closing muscles, resulting in a decrease in the excitability of the motoneurons during jaw opening. However, post-synaptic changes cannot be solely responsible for modulation of reflexes, as reflex amplitude may change while the excitability of the motoneurons remains the same.

3.4.2 Pre-synaptic inhibition

The input from the muscle spindle afferents may be affected pre-synaptically, a phenomenon known as pre-synaptic inhibition (PSI). PSI is associated with primary afferent depolarisation (PAD) of the afferent fibres. It is produced by interneurons making axo-axonic synapses with the afferent fibre terminals in the spinal cord (Rudomin, 1990). If the level of tonic PSI changes, the effectiveness of afferent input on the motoneurons may change, while the excitability of the motoneurons remains the same (Schieppati, 1987; Pierrot-Deseilligny, 1997). Thus PSI can be demonstrated when there are changes in the amplitude of the H-reflex while the level of background EMG is constant (Stein, 1995; Pierrot-Deseilligny, 1997).

Stretch and H-reflexes are often considered to be "hard-wired" with a fixed motor response to a stimulus. Pre-synaptic inhibition allows the conduction of impulses across

the synapse to be controlled, so that the afferent fibres are not passive conveyors of action potentials (Stein and Capaday, 1988; Rudomin, 1990). This allows a selective control of neuronal responses to synaptic inputs. PSI plays an important role in controlling sensory processing of information in humans and animals (Stein, 1995). For example, PSI modulates the stretch reflex in the soleus muscle during the gait cycle, allowing the soleus to be stretched before footfall without a stretch reflex being elicited (Yang and Whelan, 1993; Faist et al. 1996). There may be tonic levels of PSI on Ia afferents, and this may occur to a different extent in different muscles (Schieppati, 1987). It is possible that the difficulty of eliciting H-reflexes in some muscles (eg tibialis anterior) is due to tonic levels of PSI (Delwaide, 1973; Person and Kozhina, 1978).

PSI is difficult to record in humans because of constraints on the experimental methods that may be used. The most common method of investigating PSI in humans involves recording the modulation of the H-reflex by vibratory or electrical inputs. To ensure that these stimuli are only producing pre-synaptic changes, very short trains of stimuli should be used. The changes should be measured at a latency of 25 - 60 ms after the application of short trains of vibration, as this is where pre-synaptic changes would be expected to occur (Morin et al. 1984; Stein, 1995).

Desmedt and Godaux (1980) suggested that there was no pre-synaptic inhibition of Ia afferents to masseter, as H-reflexes and stretch reflexes are potentiated during vibration applied to the chin, rather than suppressed as is the case with the limb muscles. However, the facilitation of the H-reflexes may be due, not to an absence of pre-

synaptic inhibition, but to a different balance between post-synaptic facilitatory and pre-synaptic inhibitory mechanisms, as it is the balance between these two factors that determines how a motoneuron will respond to vibration (Ashby et al. 1980). In the jaws, the post-synaptic facilitation may be greater than the PSI. Grimwood and Appenteng (1995) examined the monosynaptic connections of masseter and temporalis spindle afferents on jaw-elevator motoneurons, and found that temporalis spindle afferents produced larger EPSPs than masseter spindle afferents. This was in part because transmission in masseter afferents was prone to failure due to tonic pre-synaptic inhibition of the masseter Ia afferents. Luo and Dessem (1999) demonstrated morphological evidence of synaptic convergences which would be capable of modulating the activity of jaw-muscle spindle afferents in rats. Modulation of jaw jerk reflexes to support stability of the mandible was established by Lobbezoo et al. (1993b), with the reflex sensitivity being larger in the absence of alternative stabilising factors such as tooth contact or visual feedback. Stimulation of trigeminal cutaneous afferents (Goldberg and Nakamura, 1977b) and the lingual nerve (Goldberg, 1972) have both been shown to result in pre-synaptic inhibition of masseter muscle spindle afferents. The source of possible PSI of the masseter Ia afferents is unclear. Lobbezoo et al. (1993b) argued against periodontal mechanoreceptors being responsible for PSI as the degree of periodontal loading hardly influenced the amount of reflex gain. This is supported by the findings of Trulsson and Johansson (1994) that periodontal mechanoreceptors reach their maximum firing rate at low force levels and therefore may not increase their contribution at high force levels.

Miles et al. (1995) suggested non-uniformly distributed tonic pre-synaptic inhibition of Ia afferents as a possible explanation for the apparent absence of functional connection between a population of Ia afferents and some masseter motoneurons. This is an interesting suggestion, as in the limb muscles PSI has been found to be equally distributed across the motoneuron pool (Zengel et al. 1983).

In summary, it appears likely that there is a degree of PSI of masseteric Ia afferents. However, this can not be demonstrated using conventional means of applying vibration, as vibration facilitates H-reflexes and stretch reflexes in masseter. If PSI of masseteric afferents is present, it may be unevenly distributed among the motoneurons.

Chapter 4. Methodological Considerations

4.1   Stimulation techniques

Stimulating the masseteric nerve to elicit H-reflexes in masseter is problematic due to the inaccessibility of the masseteric nerve, which is located 2 cm deep under the mandibular notch (Fujii, 1977). A variety of stimulation techniques have been used to stimulate the masseteric nerve, but all have had limitations.

Surface stimulating techniques have been used (Fujii and Mitani, 1973; Fujii, 1977), with the cathode over the mandibular notch and the anode either on the same or opposite side of the face, but the depth of the masseteric nerve requires that high stimulus intensities are needed. This results in the stimulation of other nerves, especially the facial nerve, and is uncomfortable for the subject. Surface stimulation also results in a very large stimulus artifact, making identification of the M-wave difficult (Macaluso and de Laat, 1995b).

Surface electrical stimulation of the masseteric nerve will also cause stimulation of cutaneous afferents. Low intensity cutaneous stimulation has been shown to cause long-latency facilitation of the H-reflex, whereas painful stimulation has an inhibitory effect (Cavallari et al. 1985). In order to determine the effect of the cutaneous component of electrical stimulation applied to evoke the H-reflex, the stimulus should be re-applied

near, but not over, the nerve being stimulated, and the reflex response in the motoneurons recorded (Semmler and Türker,1994).

Bipolar steel needles inserted 5mm apart, just below the zygomatic bone and to a depth of 20mm were used to elicit H-reflexes by Godaux and Desmedt (1975) and Cruccu (1989a; 1989b). A monopolar needle technique for producing H-reflexes in masseter has also been described (de Laat and Macaluso, 1995; Macaluso and de Laat, 1995a). In this technique, a needle is inserted as the cathode and the anode is placed on the opposite cheek. Although the bipolar and especially the monopolar techniques were successful in eliciting H-reflexes, they require the insertion of needle(s), which is uncomfortable for the subjects, especially as reinsertion of needles to obtain correct positioning may be required.

Due to the limitations of previous methods of eliciting the H-reflex, the first aim of this study will be to develop a safe, effective and comfortable method for eliciting the H-reflex in human masseter.

Stimulus durations of $0.5 - 1$ ms should be used to elicit the H-reflex, as these durations are more selective for the afferent fibres (Hugon, 1973). Due to the effects of low frequency depression, stimulus frequency should not exceed 2Hz unless the muscle is contracting voluntarily (Hugon, 1973; Burke et al 1983). In the current study H-reflexes will be investigated in the presence of voluntary contractions of the masseter, as previous research has shown that H-reflexes are only elicited in masseter during strong

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voluntary contractions (de Laat and Macaluso, 1995). However, as a new stimulating technique will be developed, the use of this technique at various contraction levels will be investigated. To prevent the effects of low frequency depression, stimulation rates below 1Hz will be used.

4.2 Recording techniques

Surface EMG is usually rectified and averaged over a number of trials to determine the reflex response of a muscle. However, interpreting the surface EMG can be misleading, as it is often not possible to determine whether the peaks and troughs represent excitation or inhibition of the underlying motoneuron pool (Widmer and Lund, 1989). Additionally, surface EMG can give no information about the behaviour of individual motor units. High threshold and low threshold motor units may respond differently to a stimulus (Garnett and Stephens, 1980), but this can not be detected using surface EMG. Recording H-reflexes in masseter using surface EMG is made difficult due to the short latencies of the M-wave and H-reflex, as they cannot always be clearly separated (Macaluso and de Laat, 1995a). Reflex latencies are also difficult to record from surface EMG recordings (Miles, 1999). These limitations of surface EMG can be overcome by recording from motor units (Miles, 1997), thus in the current study H-reflex responses in motor units will be recorded using bipolar single motor unit (SMU) electrodes.

4.3   Analysis of H-reflex responses

4.3.1   Peristimulus Time Histogram

When a neuron is firing rhythmically, the effects of an afferent volley on that neuron can be determined from the way in which the volley alters the neuron's probability of firing. This can be expressed as a peri-stimulus time histogram (PSTH). Excitation of a motor unit will result in a peak in the PSTH, whereas inhibition will result in a trough. The shape of the PSTH can be considered as the first derivative of the shape of the post-synaptic potential for excitatory inputs (Ashby and Zilm, 1978). The area under the PSTH peak has been shown to represent to underlying EPSP amplitude both theoretically (Ashby and Zilm, 1982b; Awiszus, 1992) and experimentally (Fetz and Gustafsson, 1983). However the PSTH profile cannot be used to provide a description of the falling phase of the synaptic potential (Ashby and Zilm, 1982a).

4.3.2 Cumulative sum

Ellaway (1978) described a mechanism to facilitate the detection of small changes in the PSTH that may otherwise be obscured by random fluctuations in bin counts. The cumulative sum (CUSUM) presents the cumulative change in the discharge probability evoked by the stimulus. The mean prestimulus firing of the motoneuron (k) is calculated, and this is then subtracted from the firing level in each bin, and the sum of these values is added cumulatively. The resultant curve shows trends indicating changed levels of discharges.

The latency of an H-reflex can be determined from the CUSUM of the PSTH. The raw latency from the stimulus to the onset of the steep increase in the CUSUM consists mainly of the conduction time from the stimulus to the motoneuron, the synaptic delay and the utilization time (Fetz and Gustaffson, 1983) the conduction time from the motoneuron to the muscle and the intramuscular delay time: which depends on the position of the electrode within the motor unit (Awiszus and Feistner, 1995). For this reason, the latency of the H-reflex, recorded from the CUSUM, depends on the positioning of the intramuscular electrode. To correct the latency of the H-reflex, the interval between the commencement of activity in the motor unit and the recording of the trigger spike in the MacroRep should be subtracted from the H-reflex latency determined by the CUSUM (Awiszus and Feistner, 1995).

As the CUSUM could deviate from the baseline by chance, especially over extended time periods, Davey et al. (1986) provided a derivation of the variance of the CUSUM based on the theory of stochastic point processes. The deviations of the CUSUM were found to be characterised by variance (V), which was a function of delay from the reference point (t), number of trials (n) and mean prestimulus interspike interval (I). Thus $V=(nt)/I$. Using these techniques, positive and negative significance curves are produced. A judgement can be made about the meaningfulness of the deviations of the CUSUM: if the CUSUM crosses the significance limits then a reflex response is accepted as being present.

The amplitude of the reflex response can be recorded from the size of the displacement of the CUSUM from the baseline level. The amplitude of the rising phase of the CUSUM provides an indirect estimate of the EPSP amplitude in the investigated motoneuron (Awiszus and Feistner, 1995). However, care must be taken when using the amplitude of the CUSUM to indicate size of the EPSP, as it may underestimate the size of the EPSP in small motoneurons, and overestimate the size of the response in large motoneurons, due to the greater after-hyperpolarization potential in small motoneurons (Burke, 1981).

4.3.3 Probability of a Successful Reflex Response

Semmler and Türker (1994) described a method of indicating the size of the H-reflex response. The number of extra counts in the CUSUM occurring at H-reflex latency is normalised to the number of stimuli applied. This gives the probability of a successful reflex response (PSRR). PSRR values can range from zero to one. A PSRR of one indicates that every stimulus results in a reflex response in the motor unit, whereas a PSRR of zero indicates that there was no response to the stimulus.

4.4 Subjects

Although jaw reflexes may be elicited in older subjects, the occurrence of the jaw-jerk and its amplitude are decreased in older subjects (Kossioni and Karkazis, 1994a; Kossioni and Karkazis, 1998). Reduced amplitude and longer latency of the H-reflex in

older subjects has also been demonstrated in soleus (Sabbahi and Sedgwick, 1982). Thus subjects aged 45 years and under were selected in the following studies.

Kossioni and Karkazis (1994b) investigated the influence of gender on the jaw-jerk reflex and found that the reflex latency was significantly shorter and the amplitude significantly higher in females. Therefore in the current study the gender of the subjects will be recorded, and the possible effects of gender on the H-reflex responses will be investigated. Kossioni and Karkazis (1993) investigated the effects of the menstrual cycle on jaw-jerk reflex behaviour but found no consistent trends, so all females under 45 years will be included in these studies.

As the effects of lateral tilting (Aiello et al. 1992), head rotation (Anson and Kasai, 1995; Macaluso et al. 1996) and posture (Hayashi et al. 1992; Koceja et al. 1995) on H-reflex behaviour have been clearly demonstrated, care will be taken to ensure that posture of the head and neck of the subjects are consistent in these studies.

As increasing activity in the jaw-closing muscles has been shown to increase the H-reflex in temporalis (Graven-Nielsen et al. 1998), it is necessary to control and record the level of background activity in masseter during experiments to study the H-reflex.

Chapter 5.   Eliciting and Recording the H-reflex in Masseter

5.1   Introduction

Previous methods of eliciting the H-reflex in masseter have utilised surface techniques (Fujii and Mitani, 1973; Fujii, 1977), bipolar needles (Godaux and Desmedt, 1975) or monopolar needles (Macaluso and de Laat, 1995a). Surface techniques are limited by the depth of the masseteric nerve, while needle techniques can be uncomfortable for the subject, particularly if re-insertion is needed to establish the best stimulating position. Once needle electrodes are inserted, fine lateral movements of the electrodes in relation to the masseteric nerve are not possible, which may necessitate repeated insertions to achieve satisfactory results. In addition, distortion of the needle electrodes during jaw movements is possible. Therefore, an aim of this study was to develop a new stimulating technique for the masseteric nerve that is non-invasive, flexible and effective for producing H-reflexes in masseter.

The H-reflex represented in motor units has not previously been studied in the jaw-closing muscles, although this technique has been used extensively to investigate the reflex control of the limb muscles. Previous investigations of the H-reflex in the masseter have relied on surface or subcutaneous multi-unit EMG (Fujii and Mitani, 1973; Godaux and Desmedt, 1975; Fujii, 1977; Cruccu et al. 1989b; Macaluso and de Laat, 1995a). However, the short latencies of the M-wave and H-reflex in masseter (about 2 ms and 6 ms respectively) make these responses difficult to separate from each

other and from the stimulus artifact using surface recording techniques (Macaluso and de Laat, 1995a). Recording the H-reflex in motor units is clearer, as the action potentials are all-or-nothing events that are not affected by the stimulus artifact. Also, when using surface EMG or multi-unit needle electrodes, information is gathered from a range of motoneurons and it is not possible to ascertain the effect upon individual motoneurons. Thus, another aim of this study was to investigate H-reflexes recorded in individual motor units in human masseter, using inserted wire electrodes.

5.2 Materials and Methods

Eighteen healthy volunteers (9 males, 9 females, age range 20 - 44 years) participated in the experiments. In this study and subsequent studies described in this thesis, all subjects gave their informed consent and ethical approval was obtained from the University of Adelaide Human Research Ethics Committee.

In all experiments described, in this and subsequent chapters, subjects were seated comfortably in a dental chair. Experiments usually lasted for about 3 hours. The posture of the head and neck were maintained in a neutral position and background noise was low.

5.2.1 Stimulating electrodes

The anode and cathode were incorporated into a U-shaped stainless steel frame, shown in Figure 4. The frame was held in position in the mouth by a cast of the subject's

teeth constructed of dental impression material (Formasil®), which was moulded to the shape of the subjects' teeth and attached to a flat stainless-steel bite-plate near the anode. The bite plate covered the entire occlusal surfaces of the left upper and lower teeth. The bite-plate and impression material maintained the bite position, which is
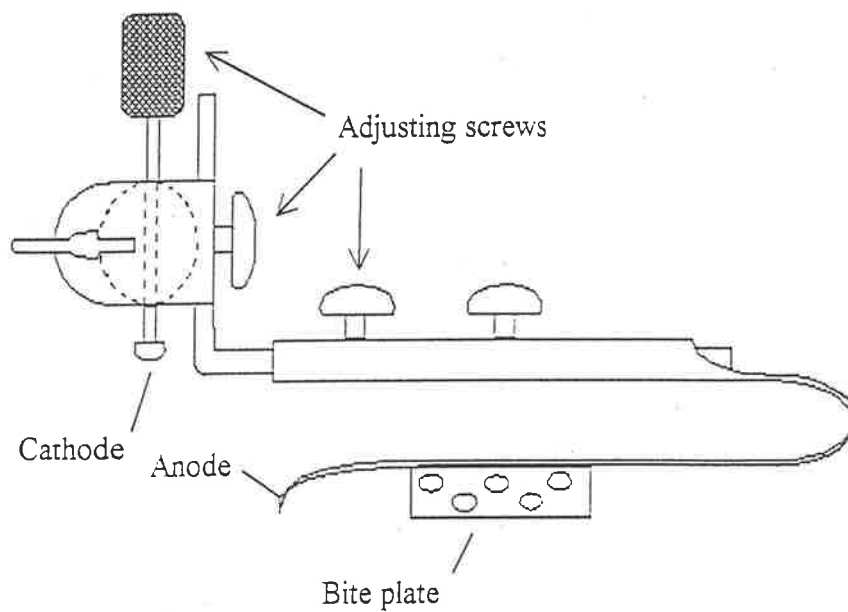
Figure 4. Transmuscular Stimulating Frame

Transmuscular stimulating frame used for application of the anode and cathode. One end of the frame was inserted into the subject's mouth so that the anode was located against the medial aspect of the left masseter, posterosuperior to the upper second molar. The position of the anode was maintained by the biting plate, which was held between the molars. Dental impression material was attached to the bite plate to allow accurate molding to the subject's teeth. Using the adjusting screws, the cathode could be moved with six degrees of freedom and then fixed in place. The cathode was positioned so that it pressed firmly into the mandibular notch.

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necessary as altering the bite position changes the firing pattern of the motoneurons (Eriksson et al. 1984). The cathode position could be finely adjusted to apply the cathode firmly into the mandibular notch.

When the jaw-closing muscles were relaxed, the bite plate rested comfortably between the teeth. Applying the cathode to the mandibular notch and the anode to the inside of the mouth allowed for transmuscular stimulation of the masseteric nerve.

5.2.2 Recording electrodes

One Teflon®-insulated bipolar silver wire single motor unit (SMU) electrode and a single Macro electrode were inserted to a depth of approximately 2 cm into the anterior inferior portion of left masseter using one 25G needle. This placed the wire tips into the deep anterior masseter (Weijs and Hillen, 1986). The insulation was stripped from the terminal 15mm of the inserted Macro electrode: the indifferent Macro electrode was attached to the right ear lobe. The SMU electrode wires were completely insulated except for the tip. The needle was withdrawn leaving the three wires in the belly of the muscle. The EMG signal from the SMU electrode was filtered at 200Hz - 5kHz and Macro at 50Hz - 5kHz. Bipolar surface electrodes were also placed over the anterior masseter on the left side of the face. These were used to record the surface H-reflex response to the stimulus, and also to give the subject feedback about the level of EMG activity in masseter. In all experiments, grounding was achieved by means of a lip-clip electrode (Türker et al. 1988). The experimental set-up is shown in Figure 5.

5.2.3 Protocol

Maximum voluntary activity (MVA) in masseter was recorded by the rectified, filtered (DC - 1 Hz) surface EMG of the left masseter while asking the subject to bite as hard as possible. The maximum of three attempts was accepted. The subject was then given visual feedback of the level of EMG activity, and asked to produce about 10% of masseter MVA. The anode and cathode were then positioned and the stimulus applied. A Digitimer Ltd. Isolated Stimulator (Model DS2) with a constant current device was used to apply the stimulus. A stimulus with pulse width of 1 ms was applied randomly at a frequency of 0.2 - 0.5 Hz. The position of the cathode and intensity of the stimulus were adjusted until an H-reflex was observed in the Macro EMG and the surface EMG, then the stimulus position was fixed. While the masseter activity level was maintained at about 20% of MVA, the effect of altering stimulus intensity on M-wave and H-reflex amplitudes was recorded. Stimulus intensity was increased in increments of 1 volt: 10 stimuli were applied at each intensity. This procedure was then repeated with the masseter muscle relaxed allowing recruitment curves of the masseter H-reflex to be plotted.

The subjects were then asked to control the discharge of one masseter motor unit at a steady frequency. Subjects gradually increased masseter activity from about 10% MVA
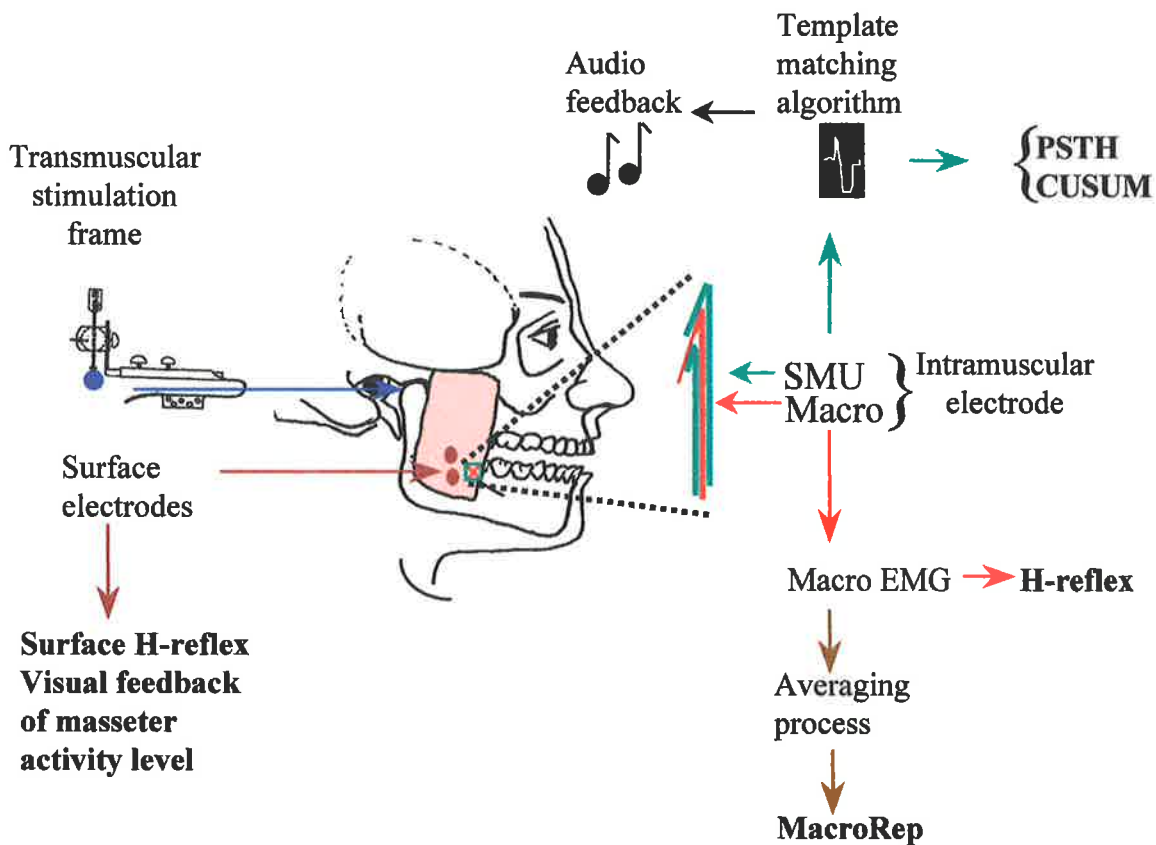
Figure 5. Experimental Set-Up for Eliciting and Recording H-reflex in Masseter.

The transmuscular stimulating frame was used to apply the stimulus, with the cathode applied against the mandibular notch. Masseter responses were recorded using surface and intramuscular electrodes. The EMG signal from the surface electrodes was used to determine the activity level in masseter and to record an H-reflex in masseter. The intramuscular electrode consisted of a pair of bipolar single-motor unit (SMU) electrodes and a single Macro electrode. The subjects received audio feedback of the firing of a motor unit, which was identified by a template-matching algorithm. Recognition pulses from the template-matching algorithm were used to construct PSTHs from which CUSUM of motor unit firing was calculated. The H-reflex in the Macro EMG was recorded, and the Macro EMG was averaged, with the firing of the motor unit as a trigger, to determine the MacroRep of the SMU

until a motor unit was observed firing on an oscilloscope screen display. Action-potential recognition on-line was achieved using a template-recognition program (SPS-8701: Signal Processing Systems, 23 Airlie Avenue, Prospect, South Australia). With the help of audio feedback of motor unit firing, subjects were able to voluntarily regulate the firing rate of a particular motor unit so that it fired at a steady frequency, of between 12 and 18 Hz. While the motor unit was firing steadily, the masseteric nerve was stimulated. The position of the cathode and intensity of the stimulus were adjusted until an H-reflex was apparent in the Macro and surface EMG, with minimal M-wave. The H-reflex was differentiated from the M-wave by the latency of the response; about 6 ms for the H-reflex and 2 ms for the M-wave. The effect of an increase in masseter contraction was also used to differentiate the responses, as an increase in masseter activity increased the H-reflex size without changing the size of the M-wave. The H-reflex was differentiated from an F-wave by determining the effect of reducing the masseter activity level, as this should decrease the H-reflex without changing the F-wave. Stimulation was continued until the subject was no longer able to continue to fire the unit regularly. The results from motor units that could not fire for at least 200 stimuli were discarded. Surface EMG, Macro EMG and motor unit activity of masseter were recorded on a videotape for off-line analysis.

5.2.4 Analysis

Surface and Macro EMG recordings were averaged (n = 10) at each stimulus intensity, with the averaging process triggered by the stimulus. From the averaged record, the

amplitude of the H-reflex and M-wave were determined, and were plotted against stimulus intensity.

The shape of the motor unit action potential was recognised using the template-matching algorithm (SPS-8701), which sent out a recognition pulse to another computer whenever it matched the shape of an action potential. The reflex response of only one motor unit was determined off-line. The chosen unit was always the one with the largest action potential. The motor unit potentials were filtered to identify the shape and the amplitude of the unit of interest and to depress the amplitudes of other units. This process reduces the chances of superimposition of other unit potentials with the potential of the unit of interest at the H-reflex latency. Such superimposition can interfere with the recognition of the unit potential at the M-wave or H-reflex latencies by the template-matching program, and hence prevent the identification of a reflex response. A PSTH of the motor unit firing was constructed from the recognition pulses from the template-recognition program on the computer. The bin width of the PSTH was 250μs. CUSUM of the PSTH was produced, and the take-off point in the CUSUM was used to calculate the H-reflex latency in the motor units. As deviations in CUSUM can occur over time by chance, significance curves were constructed (according to the methods of Davey (1986)). If the CUSUM crossed the significance lines, then an H-reflex was accepted as present.

The latency of the H-reflex measured in motor units will be affected by the position of the recording electrode within the motor unit (Awiszus and Feistner, 1993). To

accurately determine the latency of the H-reflex, the Macro EMG was triggered by the firing of the motor unit. The Macro EMG was averaged by at least 400 triggers to produce the representation of the unit in the Macro EMG (MacroRep). The time between the onset of the Macro EMG and the trigger spike was used to estimate the intramuscular delay. This was then subtracted from the latency measured by the motor unit. The bin width for the MacroRep was 0.1ms, allowing for accurate correction of H-reflex latency.

The latency of the gross M-wave and H-reflex could be determined using both the surface EMG and the Macro EMG. The Macro recording was more useful as the stimulus artifact was smaller and the M-wave and H-reflex were more easily separated.

5.3   Results

The H-reflex in masseter was present in the Macro EMG in 15 subjects. In 3 subjects, H-reflexes could not be produced even when biting levels were increased to 40% of MVA. The results are summarised in Table 2. The H-reflex and M-wave were often difficult to differentiate from one another in the surface EMG, due to their proximity to the stimulus artifact and to each other. This made determination of reflex latency difficult. Where clear separation of the responses was achieved (6 experiments), onset latencies were determined. Mean M-wave latency was 2.1 ms (range 1.8- 2.4 ms) and mean H-reflex latency was 6.4 ms (range 5.9 - 7.2 ms). The H-reflex and M-wave were clearly separable in the Macro EMG, as shown in Figure 6. H-reflex latency in the

Subject	Age	Sex	Surface EMG		Macro EMG		Motor Units	
			H-reflex latency (ms)	M-wave latency (ms)	H-reflex latency (ms)	M-wave latency (ms)	Raw H-reflex (ms)	Corrected H-reflex (ms)
1	21	M			7.2	2.3	10.5	8.3
							7.5	6.9
2	24	M	7.4	2.4	6.1	2.3	8.3	6.5
							7.5	6.1
3	40	M			6.9	2.2	9.3	7.2
							8.0	6.5
4	20	M	6.3	2.3	6.0	2.1	8.5	6.9
							7.8	6.5
5	26	M	6.0	2.0	5.9	2.0	7.8	6.9
							7.5	5.9
6	38	M			5.8	1.9	10.0	8.5
							8.8	6.8
7	29	M			6.1	2.4	9.1	7.7
							8.8	6.6
8	23	M			6.2	2.6	8.5	7.9
							7.8	6.5
9	25	F			6.2	2.0	8.8	6.4
							7.5	6.2
10	44	F	6.0	1.9	5.8	2.1	7.7	6.0
							7.3	6.3
11	33	F			6.5	2.2	7.8	6.1
							7.5	7.1
12	36	F			6.7	2.3	8.8	8.0
							8.5	7.5
13	24	F	6.3	1.8	6.0	2.4	8.8	7.9
							8.0	7.1
14	22	F			5.9	2.0	7.8	7.0
							7.5	6.4
15	21	F	5.9	2.0	5.8	2.1	8.0	7.52
Mean			6.4	2.1	6.2	2.2	8.3	7.0

Table 2. H-reflexes Recorded in Surface EMG, Macro EMG and Motor Units.

The latencies of the H-reflex and M-wave in surface EMG and Macro EMG are shown, as well as the raw and corrected H-reflex latencies from individual motor units. From the surface EMG, H-reflexes and M-waves were only clearly separable in 6 subjects, but were clearly seen in all subjects using Macro EMG.
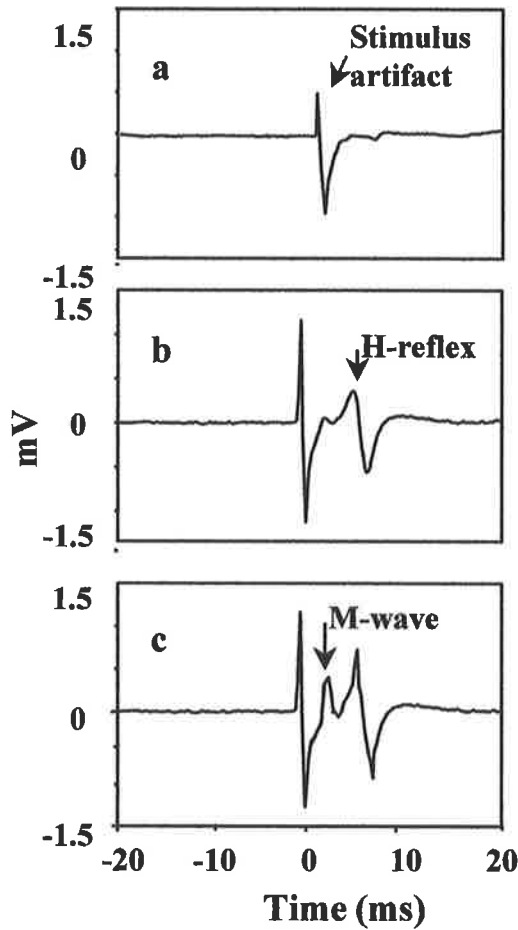
Figure 6. Masseter H-reflex and M-wave in Macro EMG

The H-reflex were clearly seen and separable in the Macro EMG. (a) The stimulus intensity was inadequate to produce a response, and only the stimulus artifact is visible. When stimulus intensity was increased (b) an H-reflex was apparent, with a latency of 5.1 ms. As stimulus intensity was further increased (c) the H-reflex amplitude increased and an M-wave was seen to develop at a latency of 2.0 ms.

Macro EMG was 6.2 ms (range 5.8 - 7.2) and M-wave latency was 2.2 ms (range 1.9 - 2.6 ms). The slightly lower reflex latencies in the Macro EMG than in the surface EMG are probably due to the clearer recognition of the onset of the responses in the Macro EMG. Contrary to the findings of Kossioni and Karkazis (1994b) there were no differences in mean H-reflex latency between the male and female subjects (mean for males 6.2 ms; mean for females 6.1 ms).

H-reflex and M-wave amplitude recorded in the Macro EMG were plotted against stimulus intensity. In twelve experiments the H-reflex amplitude decreased as stimulus intensity was increased, but in only eight experiments was it possible to completely eradicate the H-reflex by increasing stimulus intensity. In twelve experiments, H-reflexes could not be produced in the absence of a voluntary contraction. In the remaining three experiments the H-reflex could be produced at a lower stimulus intensity when the subject was biting at 20% of masseter MVA than when the masseter was relaxed. Figure 7 shows the amplitude changes in the H-reflex and M-wave recorded in the Macro EMG during two experiments. In one of these experiments, (a), the H-reflex was extinguished at higher stimulus intensities. In the other, (b), H-reflex amplitude decreased, but the reflex did not disappear even at high stimulus intensities. In both (a) and (b), the H-reflex appeared at a lower stimulus intensity than the M-wave, then the H-reflex amplitude increased in size and then gradually decreased with increasing stimulus intensity. The M-wave gradually increased with increasing stimulus intensity. The H-reflex was produced at a lower threshold stimulus intensity when the subject was biting at 20% masseter MVA than when relaxed. H-reflex amplitude was small compared to
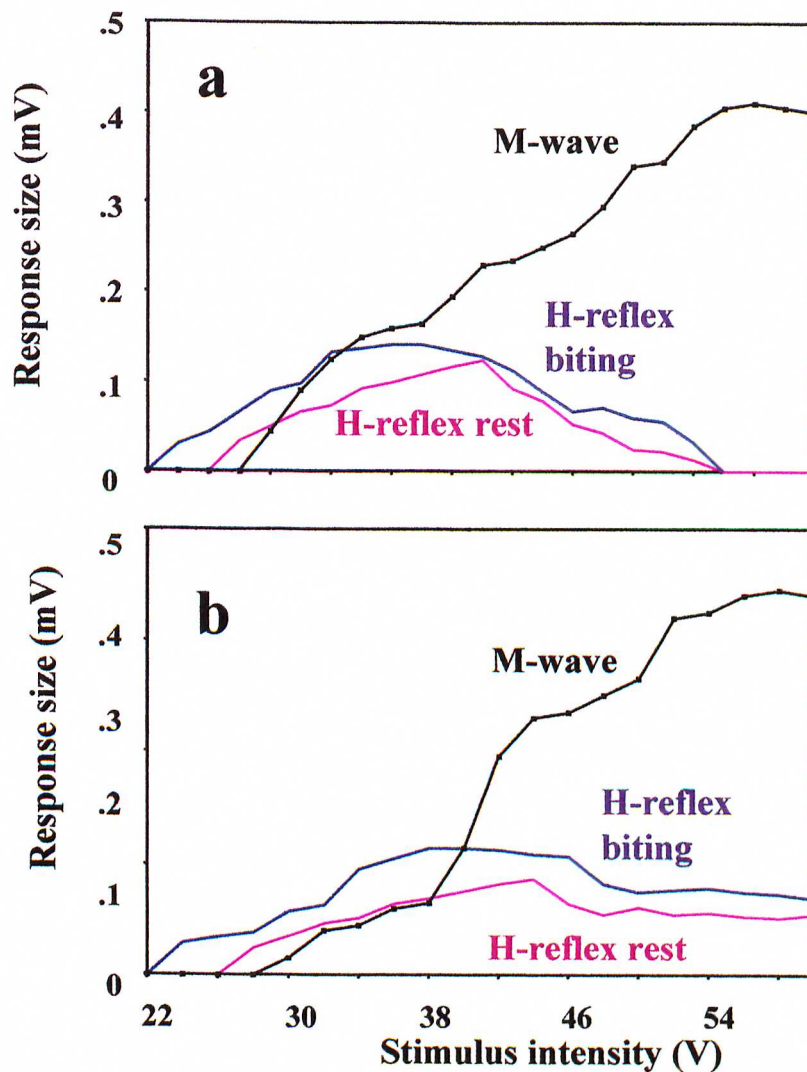
Figure 7. H-reflex and M-wave Recruitment Curves in Masseter

The H-reflex or M-wave amplitude (ordinate) is plotted against stimulus intensity (abcissa). When the subject maintained a voluntary contraction (H-reflex biting), the H-reflex was larger and was present at a lower stimulus intensity than when the subject was at rest. **(a)** With increasing stimulus intensity, H-reflex size decreased and an M-wave developed **(b)** As stimulus intensity increased, H-reflex size gradually increased and an M-wave became apparent. The H-reflex did not disappear completely with increasing stimulus intensity.

maximum M-wave amplitude, ranging from 12 - 25 % of maximum M-amplitude (mean 17%).

H-reflexes were recorded in 29 motor units during the 15 experiments. Figure 8 shows an example of the PSTH and CUSUM produced in a motor unit. The significance curves clearly show that the deviation in the CUSUM is indicative of an H-reflex occurrence. The probability of a successful reflex response (PSRR) indicates the strength of the reflex in responses per stimulus. Thus a PSRR of 0.2 indicates that the motor unit responded at H-reflex latency for two out of every ten stimuli. This figure shows that the latency of the H-reflex, as shown by the take-off point of the CUSUM, was 10.5 ms. Figure 9 shows the averaged Macro EMG representation triggered by the firing of the motor unit (n=400) illustrating that the earliest firing of the muscle fibres belonging to the tested motor unit occurred 2.2 ms earlier than that recorded by the single fibre electrode. Thus the corrected latency for the H-reflex produced in this unit was 8.3 ms.

H-reflex latencies in motor units, calculated from the onset of a rise in CUSUM, ranged from 7.3 - 10.5 ms (mean 8.3ms). When the latencies were corrected individually by spike triggered average of the Macro the resulting range was 5.9 - 8.5 ms (mean 7.0 ms). Table 2 summarises the H-reflexes recorded from each subject in the Macro EMG, the H-reflex recorded in the motor units and the corrected H-reflex latency in the motor units.
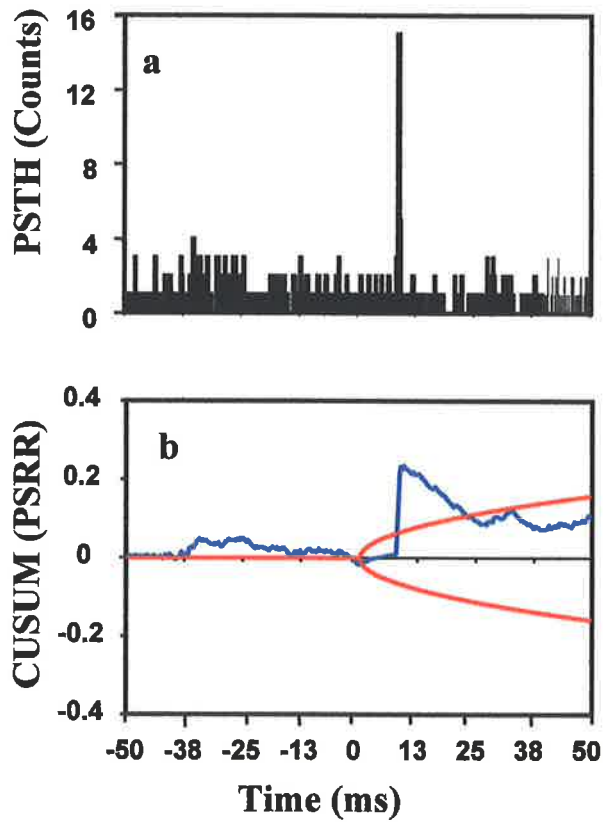
Figure 8. H-reflex in PSTH and CUSUM, with Significance Curves

(a) PSTH of SMU firing showing an H-reflex response with a latency of 10.5 ms.

(b) CUSUM of the PSTH (blue), and symmetrical significance curves (red). As the CUSUM crossed the significance lines, an H-reflex was deemed to be present.
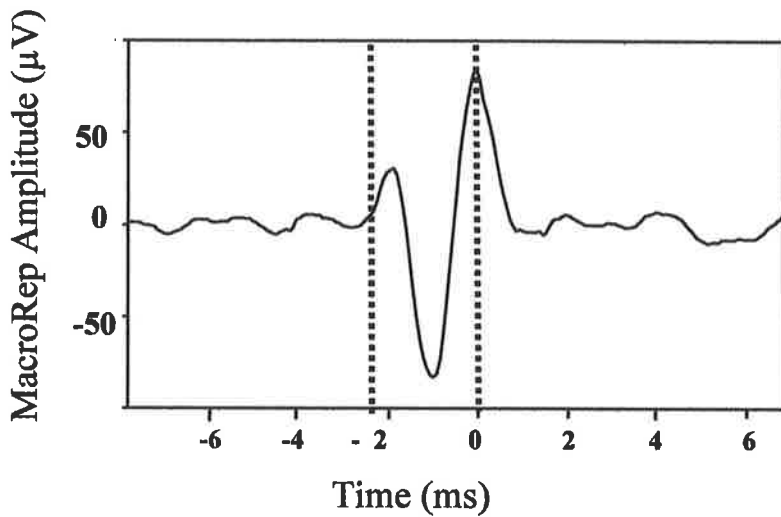
Figure 9. Representation of a Motor Unit in the Macro EMG: MacroRep

The dotted line on the left indicates the commencement of the MacroRep, whereas the dotted line on the right indicates the recording of the trigger spike. In this example, the MacroRep commenced about 2.2 ms before the trigger spike was recorded. Thus this value was deducted from H-reflex latency as measured by the CUSUM to determine the corrected H-reflex latency.

5.4 Discussion

5.4.1 Stimulating technique

The H-reflex can be successfully elicited in the surface and Macro EMG, and in motor units, using a transmuscular stimulating technique that is comfortable and non-invasive. Macaluso and de Laat (1995b) compared surface, bipolar and monopolar techniques for stimulating the motor nerve to masseter. In the monopolar technique, a needle electrode was inserted between the coronoid process and condyle of the mandible to a depth of 1.5 cm. The anode was a surface electrode placed on the opposite cheek. The bipolar technique involved inserting an additional needle 5mm posterosuperiorly to the first (also used by Godaux and Desmedt, 1975; Cruccu, 1989a). The surface technique involved a surface electrode over the mandibular notch and an anode on the opposite cheek. Surface electrodes were used to record from masseter and anterior temporalis. All three techniques were found to be effective in stimulating the mixed masseteric nerve, but the monopolar needle technique was found to be more stable than the bipolar technique and to produce a greater amplitude response more easily. Fujii and Mitani (1973) and Fujii (1977) used surface electrodes to stimulate the masseteric nerve. Electrode placement in these studies was superficial, with the cathode placed over the mandibular notch and the anode on the skin of the same side of the face, several centimetres away. Such electrode placement is not ideal for stimulation of the masseteric nerve, which is situated at a depth of about 20mm (Godaux and Desmedt, 1975). Furthermore, the surface stimulation technique also activated cutaneous afferents and the facial nerve, leading to discomfort for the subject (Macaluso and De Laat, 1995b).

In the current study, a transmuscular stimulating technique was used to produce a stable response with little or no discomfort to the subject. The placement of the anode inside the mouth allowed the stimulation to be localised to the masseteric nerve without spreading. When stimulating a nerve it is usually necessary to manipulate the position of the cathode in relation to the nerve, in order to obtain a maximal H-reflex with a minimal M-response. This fine adjustment of the cathode position was easily achieved using the transmuscular stimulating technique. The cathode position was also reproducible throughout the extent of the experiment. The current stimulating technique allowed for accurate, reproducible stimulation of the masseteric nerve and for fine adjustment of the position of the cathode to evoke a maximal H response. Such fine lateral manipulation is obviously not possible with inserted stimulating electrodes, unless they are withdrawn and reinserted.

Macaluso and De Laat (1995c) investigated the effect of the position of the surface recording electrodes on the M-wave recorded in masseter and temporalis. They found that the optimal position for the surface recording electrodes for masseter was over the anterior and inferior part of the muscle belly, so this area was used in the current study for the insertion of the motor unit and Macro electrodes, and also for the surface electrodes.

Previous authors have reported that a voluntary contraction in masseter is required to elicit the H-reflex (Fujii, 1977; Macaluso and de Laat, 1995a). In this study, using the

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new stimulating method, H-reflexes in the absence of a voluntary contraction were elicited in some subjects, although H-reflexes were larger and easier to elicit in the presence of a voluntary contraction. However, masseter activity levels as high as those required by Macaluso and De Laat (1995a), of about 40%, were not required. The lower masseter activity levels in this study therefore reduced the fatigue experienced by the subjects.

H-reflexes were small, ranging from 12 - 25% of the maximum amplitude of the M-wave. This is similar to findings of previous authors (Godaux and Desmedt, 1975; Cruccu and Bowsher, 1986; de Laat and Macaluso, 1995; Macaluso and de Laat, 1995a) (see Table 1).

5.4.2 Recording responses

The very short latencies of the M-wave and H-reflex in masseter made clear separation difficult in the surface EMG. Clearer responses were visible in the Macro EMG, which is therefore preferable for studying H-reflexes in masseter. The motor unit technique used allows clear identification of the H-reflex and M-wave responses, and ensures that the stimulus artifact does not interfere with either of the responses. As the motor unit responses are distinct events, their responses are unambiguous and reliable. This technique will be therefore be useful in studying the distribution of excitatory and inhibitory inputs to motoneurons.

The latency of the H-reflex as measured by surface EMG and Macro EMG in this study was similar to that found by previous authors (eg Cruccu and Bowsher, 1976: de Laat and Macaluso, 1995: see Table 1). However, the motor unit latency measured from the CUSUM of the PSTH was longer. This was partly due to the delay caused by conduction of the action potential through the muscle to the tip of the motor unit electrode. When the latency was corrected using the MacroRep, the latency values found were still somewhat higher than those found previously, although the lower part of the range is similar to values found using other techniques. Smaller motor units with longer latencies are likely to be under-represented in the surface recordings used by previous authors, since the surface responses will be dominated by shorter latency units presumably belonging to high recruitment threshold motor units (Burke, 1981; Stuart and Enoka, 1983). The higher latencies occasionally found in the current study highlights the ability of this technique to record from motor units of different sizes and with different latencies.

There are a number of ways to determine that the response achieved was an H-reflex and not an F-wave, which is of similar latency (Veale and Hewson, 1973).

- An M-wave did not occur in the motor units tested (Hugon, 1973).

- The reflex increased in size when the subject contracted masseter and this would not be expected to occur with an F response (Mayer and Feldman, 1967).

- The threshold stimulus intensity required to induce the H-reflex decreased with voluntary contraction.

- The H-reflex increased in size with increasing stimulus intensity and then decreased as the stimulus was increased still further, behaviour characteristic for an H-reflex but not for an F-wave (Veale and Hewson, 1973).

5.5 Summary and Implications

In this chapter, a new stimulating technique for producing H-reflexes in masseter has been described. The transmuscular stimulating frame applied the cathode externally against the mandibular notch, and the anode to the inside of the mouth, posterosuperior to the third upper molar. This technique has proven to be comfortable and effective for eliciting H-reflexes.

H-reflex responses were recorded using surface EMG, Macro EMG and single motor unit electrodes. Surface and Macro EMG produced H-reflexes and M-waves at similar latencies to those described by previous authors, although the responses were seen more clearly in the Macro EMG. Responses were also recorded in single motor units, although the range of H-reflex latencies was larger when recorded from the CUSUM of the PSTH of motor unit firing.

A possible reason for small size of H-reflexes in masseter may be due to the effectiveness of the input from the muscle spindles onto the motoneurons. Comparing H-reflexes in large and small motor units may give an indication of the effectiveness of the Ia input onto different sized motoneurons. However, it is first necessary to establish

whether recruitment in masseter is orderly according to size during normal voluntary contractions, in order to determine whether input from muscle spindles changes this order. Previous research investigating recruitment of masseter motoneurons has used techniques for measuring muscle unit size which may be inappropriate in masseter. Therefore, the recruitment of masseter motor units in slow isometric biting will be investigated in the next chapter.

Chapter 6. Recruitment of Masseter Muscle Units in Slow Isometric Contractions

6.1   Introduction

Using various methods for determining muscle unit size, including spike-triggered averaging (STA) of force to extract twitch characteristics (Goldberg and Derfler, 1977a; Yemm, 1977a) and spike amplitude (Goldberg and Derfler, 1977a; Clark et al. 1978; Desmedt and Godaux, 1979; Lund et al. 1979), previous studies have shown that the recruitment of muscle units in the jaw-closing muscles is orderly and occurs according to the size principle. However, twitch tension measured from STA records does not provide an accurate means of establishing muscle unit size in masseter (Buchthal and Schmalbruch, 1980; Calancie and Bawa, 1990; Hannam and McMillan, 1994). Spike amplitude is affected by the proximity of the muscle fibres to the recording electrode, and thus may not provide an accurate measure of muscle fibre size (Miles et al. 1986). Yemm (1977a) used spike amplitude as an indicator of muscle fibre size, and found that muscle units were recruited in order of spike amplitude, suggesting that recruitment in masseter occurs according to the size principle. However, this result is somewhat contradictory, as the smallest muscle fibres in masseter are actually the Type II fibres (Eriksson, 1982). This may indicate that the Type II motor units, which are supplied by the larger motoneurons (Buchthal and Schmalbruch, 1980) are recruited first in masseter. Therefore, there remain some questions about whether the recruitment of

muscle units into slow voluntary isometric contractions in masseter is orderly according to the size principle.

In the limb muscles, the representation of the motor unit in the Macro EMG (MacroRep) has been shown to be a good indicator of the size of a muscle unit (Ashby et al. 1986; Dengler et al. 1989; Vogt et al. 1990; Lemon et al. 1990; Masakado et al. 1994; Jabre and Spellman, 1996). Either the amplitude or the area of the MacroRep can be used as an indicator of muscle unit size, although amplitude is the more important measure (Stålberg, 1983). Thus in this study the recruitment of masseter muscle units will be studied using the amplitude of the MacroRep of motor units as an indicator of muscle unit size.

In order to determine whether muscle unit recruitment occurs according to the size principle, the size of the muscle unit (as measured by twitch characteristics, MacroRep, or spike amplitude) is usually plotted against the force developed by the muscle. The force recruitment threshold of a motor unit is then correlated with the muscle unit size. However, this relies upon the assumption that the force contribution of the muscle under investigation, to the total force being developed, is constant. This may not be a valid assumption at joints that are moved by more than one muscle, such as the jaw. Bite force is developed by three pairs of jaw-closing muscles (masseter, temporalis and medial pterygoid) and is primarily opposed by digastric. If the contribution of the participating muscles, and the activity in the antagonist, is not consistent between biting attempts, then the force recruitment threshold of masseter motor units could be an

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unreliable indicator of motor unit size, even if it is established that recruitment occurs according to the size principle.

Therefore, the aim of the current study were:

1. To investigate the recruitment of human masseter muscle units into voluntary slow biting contractions, using the MacroRep amplitude to determine muscle unit size.

2. To investigate the stability of the contribution of masseter, anterior temporalis and digastric to a slow isometric biting task, to determine the usefulness of the force recruitment threshold in studies investigating recruitment of motor units in masseter.

6.2   Materials and Methods

Eight subjects with normal dentition and no history of temporomandibular dysfunction took part in the study.

6.2.1 Force and EMG recording

Bipolar surface electrodes were used to record surface activity in left masseter, anterior digastric and anterior temporalis. The signal from all muscles was amplified (3000 times), filtered (20Hz - 1kHz) and recorded on LabView³ (*National Instruments*) for off-line analysis. Bite force was measured using a strain gauge mounted on a bite bar, amplified (100 times) and filtered (DC - 250 Hz) and was simultaneously recorded on LabView®. The subject bit into an impression of his/her own teeth attached to the bite
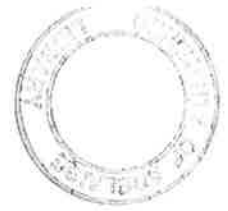
bar. The position of the head was further stabilised by using a head-rest against the forehead.

Intra-muscular activity, was recorded as described in Chapter 5, using SMU and Macro electrodes.

6.2.2 Protocol

Maximal bite force (MBF) was recorded by asking the subject to bite three times as hard as possible, for a period of 5 seconds: the greatest force was accepted as MBF. During these MBF attempts the maximal level of surface EMG (in mV) in masseter and temporalis were also recorded. Maximal digastric surface EMG activity was recorded while asking the subject to resist a strong jaw-closing force, manually applied by the researcher. The highest value of the rectified, filtered (DC-1 Hz) surface EMG of the digastric during this maximal isometric contraction, and the highest value of the rectified, filtered (DC-1 Hz) surface EMG of masseter and temporalis during the maximum bite force, were used for normalising the data collected during ramp contractions. Bite force was displayed on an oscilloscope screen, and subjects were asked to follow a force ramp, slowly developing 75, 150 or 300N of isometric jaw closing force over a period of 120 seconds. The smaller ramps allowed for detailed investigation of motor unit recruitment at low force levels, whereas the 300N ramp approached maximum for most subjects and allowed for investigation of the activity of masseter, temporalis and digastric during the development of force. Subjects were asked

to bite vertically onto the bite bars during the ramp contractions, while maintaining the head in contact with the headrest. After each ramp contraction the subjects were taken off the bite bars and rested for at least 5 minutes. Biting position was maintained between contractions by the impression material into which the subjects bit for each ramp. The order of the different size bite force ramps (ie 75N, 150N or 300N) was randomised to minimise the effects of fatigue.

6.2.3 Analysis.

The 300N ramps were used to illustrate the relationship between the bite force and the activity of the muscles. Surface EMG from masseter, temporalis and digastric was rectified and filtered (DC-1 Hz) and normalised to values recorded during maximum jaw closing and opening tasks. The EMG recordings were then plotted against force to determine the contribution of each muscle, as a percentage of its maximum, during a steadily increasing force.

The 75N and 150N ramps were used to investigate the recruitment of muscle units into slow isometric ramp contractions. Motor unit action potential recognition was achieved using the template-matching algorithm (SPS-8701). At high bite force levels, several units were activated and careful filtering was necessary to separate units. The template-matching program was set to reject action potentials that were superimposed and to register only those units that were clearly different from each other. The program also allows any superimposed spikes to be examined and separated off-line. For each motor

unit, the force recruitment threshold and masseter surface EMG recruitment thresholds were determined. These values were normalised to MBF or maximum surface EMG activity. The MacroRep of the motor units was determined as described in Chapter 5.

For each subject, the results from repeated ramps were pooled, and the relationships between force recruitment threshold and MacroRep size, and masseter surface EMG recruitment threshold and MacroRep size were calculated using Pearson's r correlation coefficient. Where the same motor unit could be identified in separate ramps performed by the subject, the force and surface EMG recruitment thresholds of the motor unit were compared in different ramps. The maximum and minimum force and surface EMG recruitment thresholds were determined for each motor unit. From this, the recruitment threshold ranges (maximum – minimum) was calculated. A paired t-test was used to determine whether there was a significant difference in the force and surface EMG recruitment threshold ranges. Statistical significance was accepted when p was smaller than 0.05.

6.3   Results

The number of ramps completed by each subject are shown in Table 3. The results from the 300N ramps were used to investigate muscle recruitment patterns of masseter, temporalis and digastric, whereas the 150N and 75N ramps were used to investigate the recruitment of motor units into masseter contractions. From the 48 ramps of 150N and 75N, 168 different motor units were identified. Of the 168 motor units, 20 could be

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Subject	300 N Ramps	150 N Ramps	75 N Ramps	Number of MUs (75,150N)	MacroRep vs Force RT: r value	MacroRep vs Surface EMG RT: r value	Repeat MUs
1	6	4	2	24	.29	.59*	
2	5	2	2	12	.33	.67*	4
3	4	4	2	20	.19	.51*	2
4	4	4	5	30	.28	.39	4
5	4	3	3	20	.27	.55*	3
6		4	3	21	.33	.56*	4
7		3	2	21	.32	.76*	3
8		3	2	20	.49*	.70*	
Total	23	27	21	168			20

Table 3. Biting Ramps Produced by Subjects, and Motor Units Identified.

The number of 300N, 150N and 75N ramps produced by each subject, and the number of different motor units identified during the 150N and 75N ramps, are shown. The relationship between the amplitude of the MacroRep and the force/surface EMG recruitment thresholds of the identified motor units (MUs) is indicated in terms of the Pearson's correlation co-efficient. The significance of the correlation co-efficient is indicated: * $p < 0.05$. Repeat MUs, shows the number of motor units that were identified in at least 4 ramps.

identified repeatedly, occurring in at least 4 different ramps. For these 20 units, force and surface EMG recruitment thresholds were compared in the runs in which they were identified.

6.3.1 Instability of contribution of muscles to biting

Maximum bite force ranged from 320N to 400N (mean = 340N). Figure 10 shows the contribution of masseter, temporalis and digastric to the development of four 300N bite force ramps in one of the subjects. The surface EMG record from each muscle was rectified, filtered and normalised to maximum surface EMG recordings. The maximum bite force in this subject was 340 N. The variability in the contribution of the three muscles to the development of the 300 N ramp is clearly shown. All subjects showed similar variability in the contribution of the three muscles. Figure 11 shows the varying relationship between bite force and normalised surface EMG for left masseter, temporalis and digastric, in five subjects. Each line indicates the mean activity of 4 - 6 ramps of 300 N produced by a subject. As shown, there were large differences in the contributions of masseter, digastric and temporalis for each subject. In one subject the temporalis activity recorded during the bite ramp was greater than that recorded during the maximum bite task. In this case the normalised temporalis activity during the ramp exceeded 100%. The main antagonist to jaw closing, the digastric, was noted to be active during all contractions, its mean contribution varying between 5% and 30% of maximum.
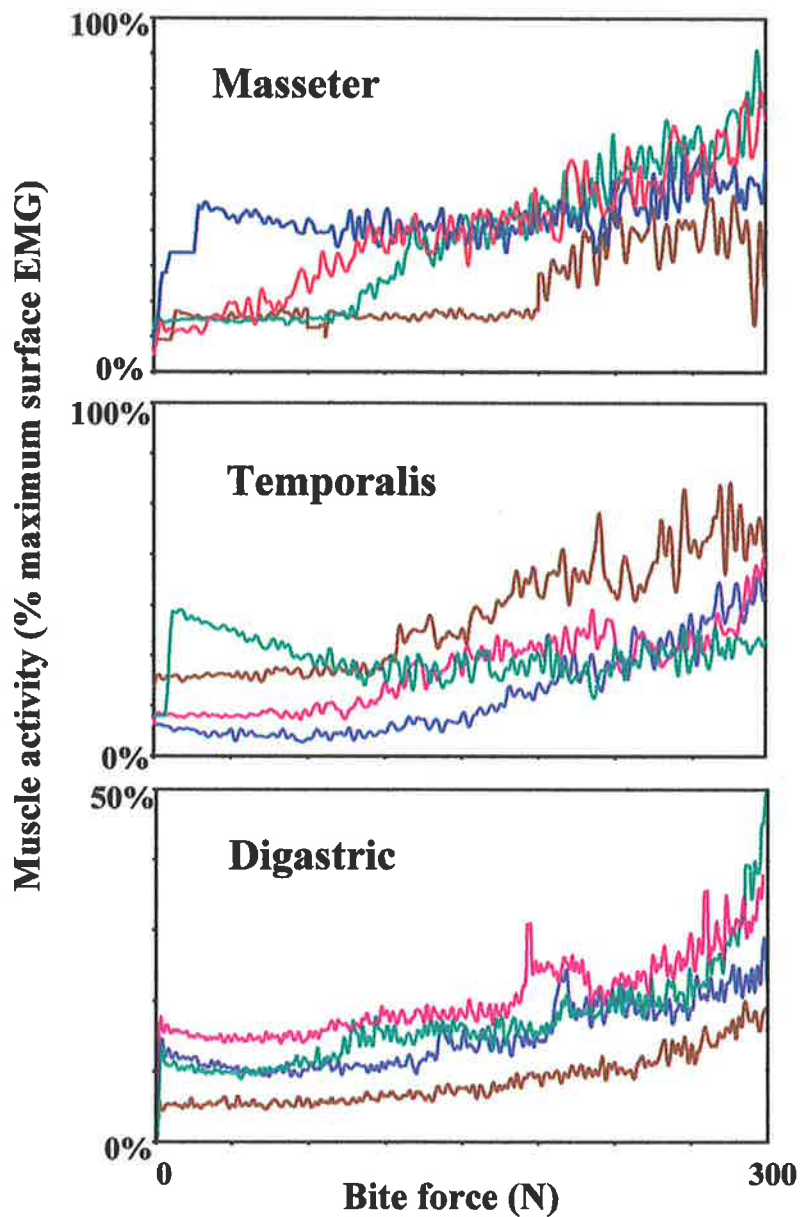
Figure 10. Variability in Muscle Recruitment During Biting in One Subject

Four separate 300N ramps produced by one subject, showing the contribution of masseter, temporalis and digastric to each ramp. The muscle activity is expressed as a percentage of the maximum muscle activity produced during maximal jaw closing (masseter and temporalis) or opening (digastric). Each ramp is shown in a different colour. Note the different vertical scale for digastric.
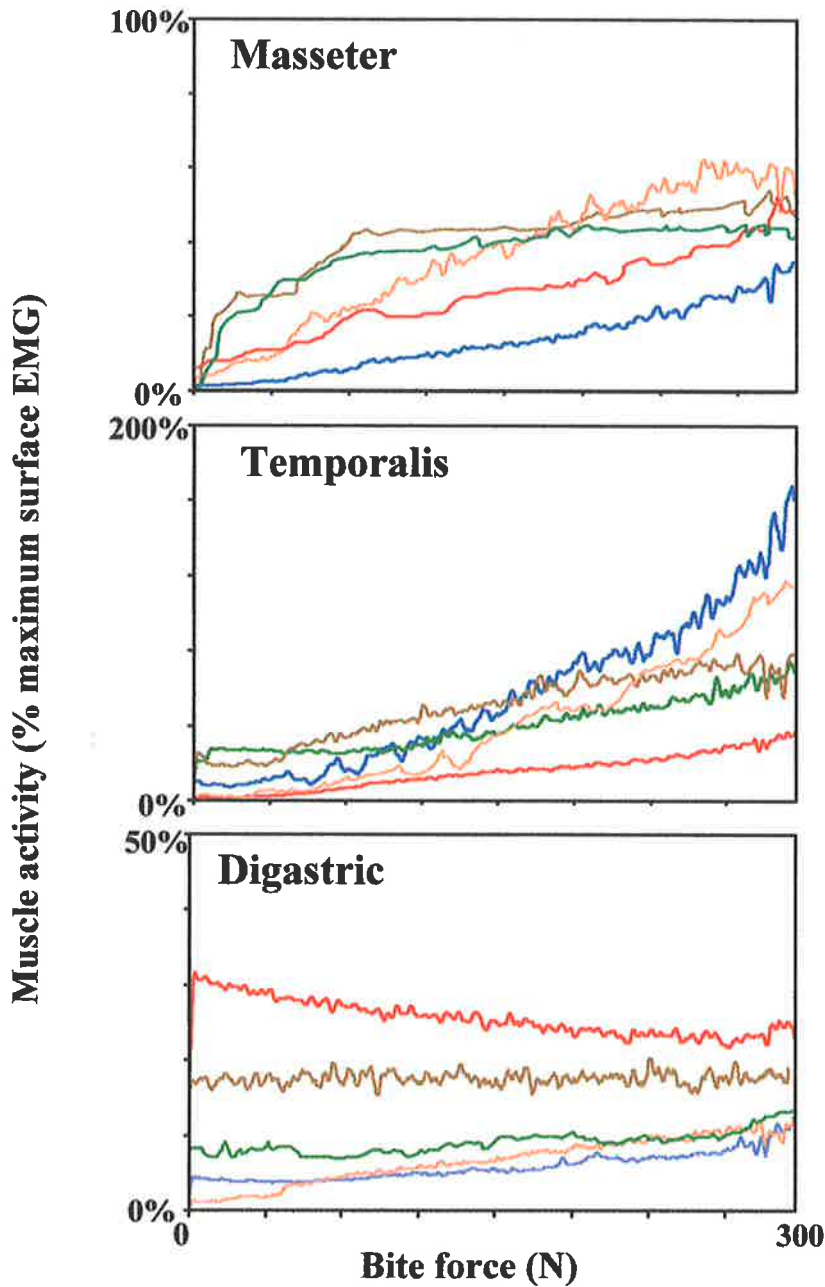
Figure 11. Muscle Recruitment Patterns During Biting in Five Subjects.

Mean surface EMG activity (as a percentage of maximum) of masseter, temporalis and digastric in 300N biting ramps in 5 subjects. Each line represents the mean of 4-6 ramps by a subject. Each colour represents a different subject. Note differences in vertical scale for temporalis and digastric.

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6.3.2 Macro representation versus force recruitment threshold and surface EMG recruitment threshold

Figure 12 shows the MacroReps of 6 motor units identified during a 150N ramp, illustrating that the motor units were recruited in order of increasing MacroRep size. When each ramp was considered individually, the motor units were recruited in order of increasing MacroRep amplitude in 90% of ramps (43 out of 48 150N/75N ramps). The MacroReps of all the motor units recorded for each individual subject, and their force recruitment thresholds, were pooled, and the relationship between force recruitment threshold and MacroRep amplitude determined. Each of the 8 subjects completed 4 - 9 150/75 N ramps, and between 12 and 25 different units were identified for each subject (Table 3). When the relationship between MacroRep size and force recruitment threshold was investigated with each subject's pooled data, there were only weak relationships found. Stronger relationships were found between pooled Macro amplitude and surface EMG recruitment threshold for each subject. An example of these relationships in the pooled data of one subject is shown in Figure 13. Figure 13(a) shows the relationship between MacroRep amplitude and surface EMG recruitment threshold for 5 ramps ($r = 0.76$, $p < 0.05$). Figure 13(b) shows the relationship between MacroRep amplitude and force recruitment threshold in the same subject. Pooling of the data points showed a weaker relationship between MacroRep size and force recruitment threshold ($r = 0.32$, $p > 0.05$).
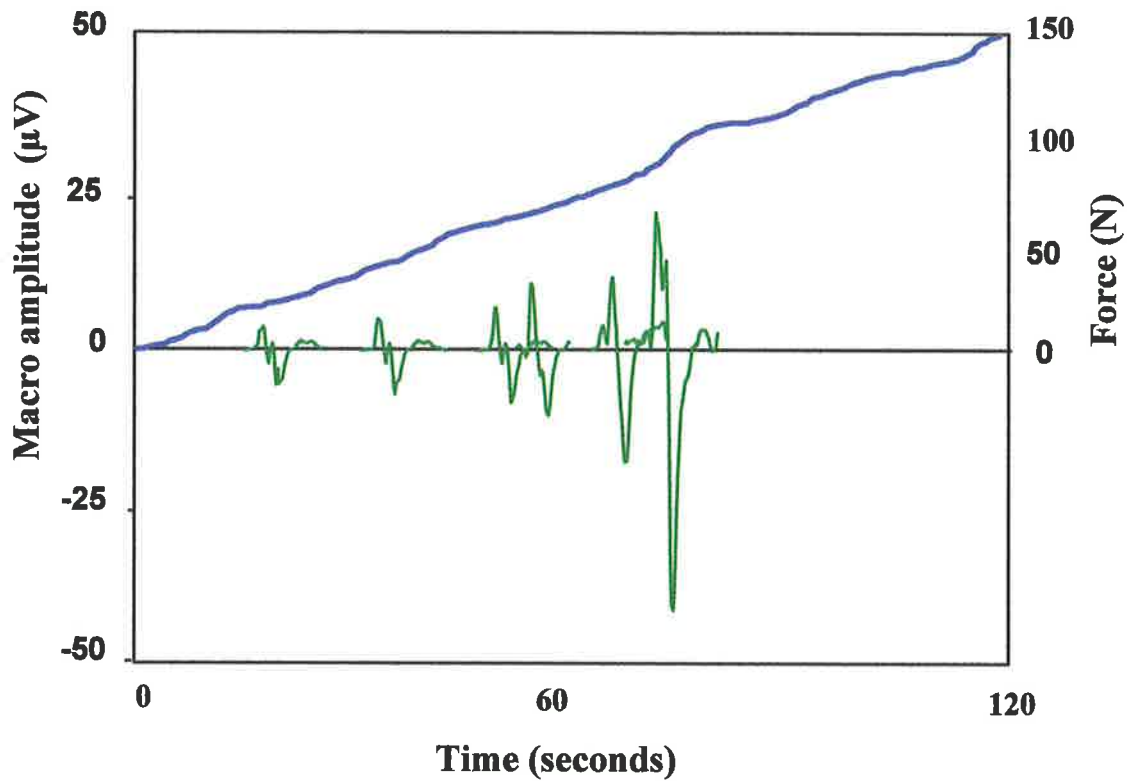
Figure 12. MacroRep Amplitude with Increasing Bite Force.

MacroRep amplitude of six masseter motor units recruited during a 150N bite ramp developed over 120 seconds. Recruitment of these motor units was orderly according to the amplitude of the MacroRep.
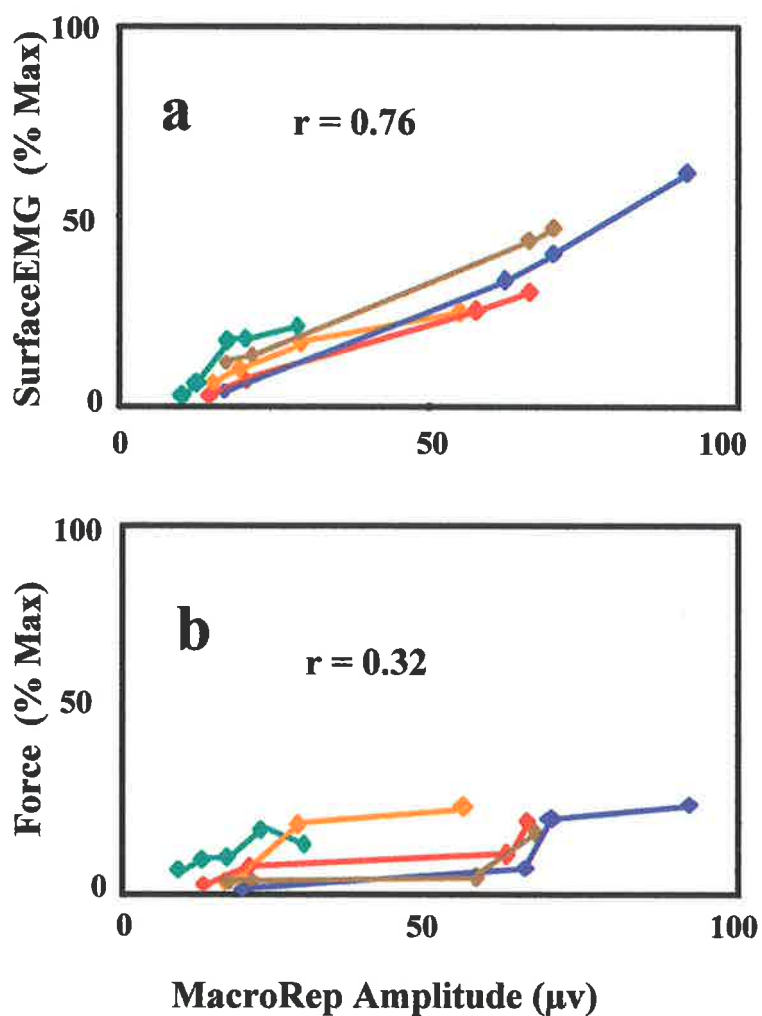
Figure 13. Relationship Between MacroRep Amplitude and Surface EMG/Force

The relationship between MacroRep amplitude and (a) % maximum surface EMG and (b) % maximum force. Five ramps performed by 1 subject, during which 21 motor units were recruited, were pooled. A significant (p<0.05) relationship was found between MacroRep amplitude and surface EMG recruitment threshold (r = 0.76), but there was not a significant relationship between MacroRep size and force recruitment threshold (r = 0.32).

6.3.3 Recruitment of motor units at different force and surface EMG levels

Figure 14 shows the number of motor units recruited at different bite force and surface EMG levels. For the 168 motor units identified from the 8 subjects, the mean force at which motor units were recruited was 10.5% of maximum bite force and 87% of the units were recruited below 19.9% of maximum bite force. The maximum force at which any additional unit was recruited was 32.5% of maximum bite force. In contrast, motor units were recruited up to 63% of maximum surface EMG, and were distributed quite evenly up to 50% of maximum surface EMG.

6.3.4 Instability of force recruitment threshold

In six subjects, it was possible to identify motor units that were recruited in at least four different ramps. To determine whether the same unit was recruited in successive ramps, the size and shape of the MacroReps were closely inspected. The tolerance and template adaptability in the template-matching program were also set to minimal levels. Only in cases where there was less than 5% change in the amplitude of the MacroRep, and no discernible change in shape, was the unit accepted as being the same unit. Twenty motor units were identified as occurring in at least 4 ramps. The force and surface EMG recruitment thresholds of these units were determined in each of the ramps. Figure 15 shows the difference between the highest and lowest recruitment thresholds for force and masseter surface EMG for each motor unit. The differences between maximum and minimum force recruitment threshold (force recruitment range) was 5.4% to 23.6% of
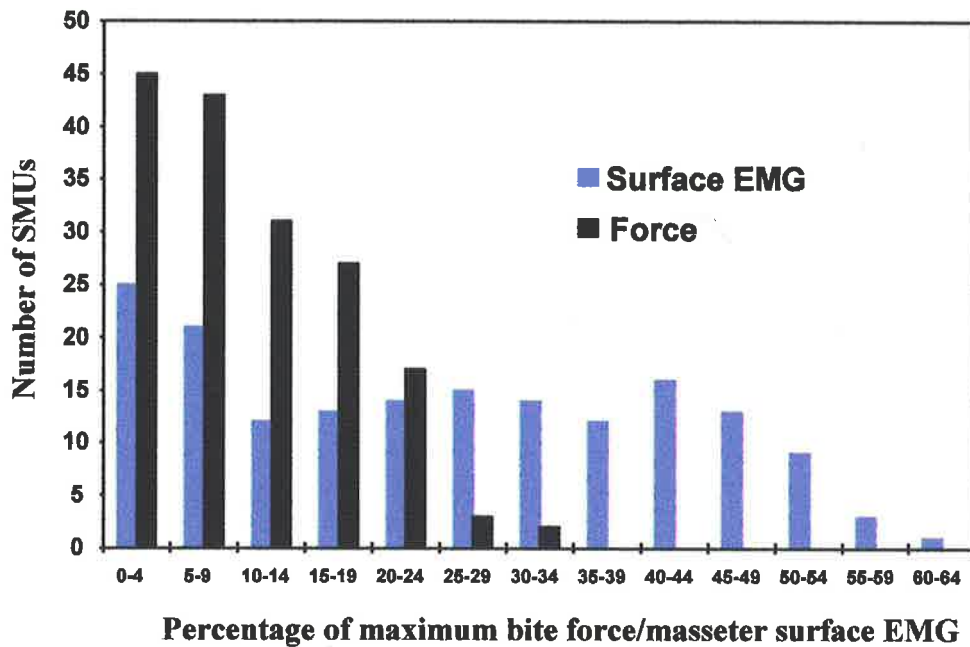
Figure 14. Motor Units Recruited at Different Bite Force Levels

Pooled data from 8 subjects, showing the number of motor units recruited at different percentages of masseter surface EMG and bite force (total motor units=168). Motor units were recruited up to 63% of maximum masseter surface EMG, but only up to 32.5% of maximum bite force.

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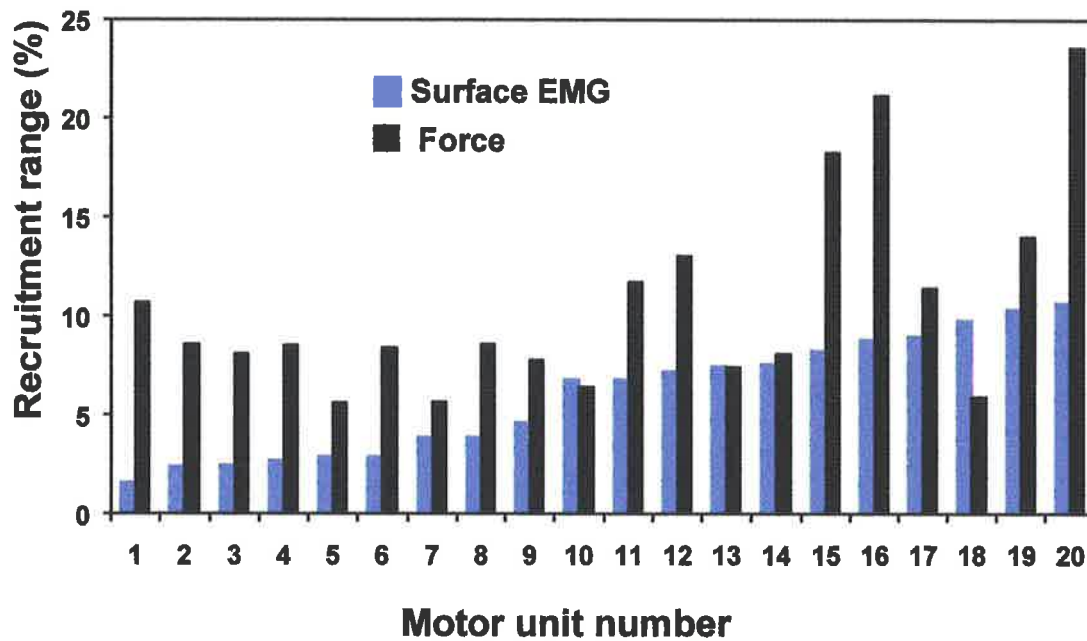
Figure 15. Instability of Force Recruitment Threshold and Surface EMG Recruitment Threshold.

For the 20 units identified in consecutive ramps, the surface EMG and force recruitment ranges (maximum - minimum) are shown, ordered according to the size of the differences in the surface EMG recruitment threshold. The force recruitment range was larger than the surface EMG recruitment threshold in 17 out of 20 motor units.

maximum force. There was more consistency in the surface EMG recruitment threshold, with the differences between maximum and minimum surface EMG recruitment threshold (surface EMG recruitment range) being from 1.6% - 10.3% of maximum force. The force recruitment range was significantly larger than the surface EMG recruitment range (t = -4.8, p <0.001).

6.4 *Discussion*

6.4.1 Variability in the contribution of muscles to jaw closing force

Jaw closing force can be produced by activity in masseter, temporalis and medial pterygoid, with the main opposing force being provided by digastric. The masticatory system has been described as mechanically redundant (van Eijden et al. 1990) as a particular jaw closing force could in theory be produced by an infinite combination of muscle forces from the participating muscles. The same situation potentially applies to movement of most joints in the body: there are few movements that are produced by only one muscle, although abduction of the index finger by the first dorsal interosseous is an important example (Zijdewind and Kernell, 1994).

In this study, all subjects showed different patterns of jaw muscle activity during a standard bite task, and these patterns altered in different ways in each subject. Even in repeated ramps during the same experiment, the contribution of various jaw muscles to the bite force changed considerably (Figure 10). In contrast to the findings of van Eijden et al. (1990), that digastric activity never exceeded 10% of maximal levels, digastric

activity in this study varied considerably, fluctuating in the range 2 - 50%. This large variability in digastric represents variable levels of co-contraction and could have required changes in activity of temporalis and masseter as the bite ramps were produced. High levels of masseter activity noted early in the ramp in some subjects could be accounted for by early activity in digastric. Van Eijden et al. (1990) stated that for forces above 50N there was a linear relationship between force and EMG in the jaw-closing muscles. However, the patterns of activity used by anterior temporalis, masseter and digastric by the seven subjects in that study varied considerably (Figure 1), consistent with the variability in the current study. Mao and Osborn (1994) found consistent ratios of masseter/temporalis activity between individuals and suggested that human subjects share similar patterns of jaw muscle recruitment. This is in contrast to the findings of the current study and previous work (Hagberg et al. 1985; Lindauer et al. 1991). The difference between the results in the current study and the findings of Mao and Osborn (1994) may relate to the different methodologies used: a ramp force in the current study and incremental 50N bite tasks in their study. Furthermore, neither van Eijden (1990) nor Mao and Osborn (1994) investigated the first 50 N of bite force: the current study showed that many of the masseter motor units were recruited in this part of the contraction.

In the current study, the activity of only the left-sided muscles was recorded. However, as the muscles of each side contribute almost equally to the production of bite force in the vertical direction (van Eijden et al. 1990) and there are no significant differences in

the muscle activity in the right and left masseter in healthy subjects (Rilo et al. 1998), the results may be extrapolated to the right-sided muscles.

In the current study a bite plate that carried the impression of the upper and lower teeth was used to maintain the relationship between the teeth on subsequent bites. Although the biting position, head posture and bite instructions were consistent, the relationship between surface EMG of masseter, anterior temporalis and digastric and the total bite force still varied considerably with repeated biting attempts. This study shows that when subjects are asked to repeat the same bite task they may use different biting strategies. If the direction of biting was more restricted, using bite bars with triaxial force transducers (van Eijden et al. 1990), the biting patterns may be less variable. Therefore, it may be suggested that the variability in activity in anterior masseter, anterior temporalis and digastric in the current study may be due to different biting strategies used on different biting attempts. Although this may provide a partial explanation, it cannot explain the large changes in activity in anterior temporalis, as van Eijden et al. (1990) found that the activity in anterior temporalis did not change greatly with changes in biting direction. Only the vertical component of biting force was measured in this study, although head position was controlled and subjects requested to use a vertical biting strategy. A triaxial feedback system such as that used by van Eijden et al. (1990) may control the biting directions used by the subjects and make the findings more consistent. However, the amount of co-contraction could still present a problem. Although it may be possible to control the direction of biting, the contributions of the jaw-closing muscles to produce the desired force cannot be controlled using force feedback alone.

The varying contributions of anterior temporalis, masseter and digastric to the total force produced have implications for the use of STA for the determination of muscle unit size in the jaw-closing muscles. If the contribution of a muscle to the total force developed is not stable, then the calculated twitch force of the unit will be unreliable. This supports the assertion (McMillan et al. 1990) that STA of force is not an appropriate technique for determining motor unit size in the jaw muscles during normal biting.

Although the activity of medial pterygoid, also a jaw-closing muscle, was not investigated, it is still clear that there is great variability in the contribution of jaw muscles to a simple task such as slow isometric biting.

6.4.2 Recruitment order of masseter muscle units

Recruitment of muscle units in single isometric biting ramps in masseter occurred according to the size principle, with the smallest units being recruited first and larger units being progressively recruited as force increased. When the order of recruitment of units during single ramps was considered, the recruitment was orderly according to size in 90% of cases.

There was a weak correlation between force recruitment threshold and muscle unit size when all the ramps by a subject were pooled. This is likely to have occurred due to the

varying levels of co-contraction and activation of the jaw-closing muscles. Masakado et al. (1994) and Jabre and Spellman (1996), found a progressive increase in MacroRep size with successively recruited units in first dorsal interosseous (FDI). There was a strong correlation between pooled data for force recruitment threshold and MacroRep in these studies, in contrast to the present study. The strong relationships found previously may have been due to abduction of the index finger being produced solely by FDI (Zijdewind and Kernell, 1994), so there was not the potential for varying contribution of different muscles that was found in the jaw muscles in the current study. Thus although in the current study pooling data between successive ramps (in the same subject) gave insignificant correlations because of changing contribution of the muscles, this was not an issue when finger abduction was investigated.

There was a strong relationship between pooled surface EMG recruitment threshold and muscle unit size in masseter. Using masseter surface EMG recruitment threshold takes into account the varying contribution of the jaw-closing muscles and varying levels of co-contraction, and should be preferred when determining the recruitment hierarchy of muscle units in a particular muscle.

In this study, only the recruitment of muscle units in the deep anterior masseter was investigated. Thus it is possible that recruitment in other parts of masseter may not occur in the same way.

6.4.3 Distribution and stability of force recruitment thresholds

The results support those cited by Hannam and MacMillan (1994) that about 50% of masseter motor units are recruited at 10-20% of the maximum force level, and Goldberg and Derfler (1977a), that 50% of masseter motor units are recruited at forces less than 4kg. In this study, 87% of the units were recruited between 0-20% of the maximum bite force.

Most units were recruited during the first part of the force ramp, up to about 30% of maximum bite force (MBF). No units were recruited above 32.5% of MBF. When surface EMG was used to indicate muscle unit recruitment, it was noticed that new units were recruited up to 63% of maximal surface EMG. This discrepancy suggests that masseter may be most active during the early part of the force ramp, as was seen in many experiments (Figures 11 and 12), where masseter activity increased early in the force ramp and then stayed relatively constant.

The force of muscle contractions are controlled by the number of units firing and their firing rate. Increases in force can be achieved by the recruitment of more units (recruitment coding), or by increasing the firing rate of those already firing (rate coding) (Broman et al. 1985). Considering only the force recruitment thresholds would suggest that masseter uses rate coding, as found in the small muscles of the hand (de Luca, 1985). However, the recruitment of motor units according to surface EMG recordings in this study suggests that masseter may use a combination of rate-coding and

recruitment for the development of slow isometric force, supporting the findings of Derfler and Goldberg (1978).

In human experiments, the force threshold at which motor units are recruited is used as the indicator of the size of the motoneuron, as this has been found to be the best indicator of cell size in animals (Heckman and Binder, 1990). However, when using force recruitment threshold, the activation of synergists and antagonists may not be taken into account. When using force recruitment threshold in the case of bite force, the relative contributions of masseter, temporalis, medial pterygoid and digastric are assumed to be constant. The present study, using a restricted slow isometric bite ramp showed that this was not the case. Hence, even if the recruitment order within the muscle stays constant, the absolute force recruitment threshold of a motor unit can change considerably, depending on the participation of that muscle in the force ramp. It is likely that this is the reason for the instability in motor unit force recruitment threshold found in this study. This indicates that force recruitment threshold is not a reliable measure for determining unit size, in one of the muscles that contribute to the total force output. There was less variability in the surface EMG recruitment threshold, as the varying contributions of the masseter to the total force developed do not affect this measurement.

Instabilities in force recruitment threshold have been noted by several authors (Romaiguere et al. 1989; Calancie and Bawa, 1990; Schmied et al. 1997), and many mechanisms have been proposed to account for this, including changes in the excitability

of the motoneuron pool (Romaiguere et al. 1989), Ib inhibition, pre-synaptic inhibition and Renshaw inhibition (Calancie and Bawa, 1990). These are unlikely explanations for the findings of the current study, especially as there was no apparent pattern to the changing contribution of the jaw-opening and closing muscles. Rapid fluctuations in the force produced by a subject attempting a ramp contraction (Miles et al. 1986) and changes in the speed of contraction (Yoneda et al. 1986; Masakado et al. 1995) are also often cited as a cause of different recruitment thresholds. The slow ramps used in this study make this an unlikely cause of the observed variability in force recruitment threshold. The visual feedback of force received by subjects allowed for the slow and smooth development of force ramps. Thus, the findings of the current study suggest that changing contributions of the synergists and varying levels of co-contraction could be major factors causing instabilities in recruitment threshold.

6.5   Summary and Implications

The contribution of anterior masseter, anterior temporalis and the anterior belly of digastric to the development of slow isometric force ramps has been investigated, and considerable variability between and within subjects was demonstrated. This variability may lead to inaccuracies when using force recruitment threshold or spike triggered averaging as techniques for determining muscle unit size in masseter.

Recruitment of masseter muscle units occurs in an orderly manner when MacroRep amplitude is used as a measure of muscle unit size. Due to the variations in contribution

of masseter, temporalis and digastric to the development of force, the surface EMG recruitment threshold has been shown to be a more accurate and reproducible indication of muscle unit size than force recruitment threshold.

MacroRep is used as a measure of muscle unit size. To determine whether recruitment occurs according to the size principle, it is necessary to determine whether motoneuron recruitment occurs in order of increasing size. The relationship between MacroRep and motoneuron size in masseter has not been established, thus until this relationship is determined it is not possible to demonstrate the order of motoneuron recruitment. Thus the next chapter considers the relationship between MacroRep amplitude and motoneuron size in human masseter.

Chapter 7.   Use of MacroRep for Inferring Relative Motoneuron Size in Masseter

7.1   Introduction.

Masseter muscle units have been shown to be recruited in order of increasing MacroRep amplitude (Chapter 6), indicating that recruitment of muscle units is from smallest to largest. Muscle unit size is an indicator of motoneuron size in the limb muscles, as larger motoneurons supply motor units composed of many large Type II muscle fibres (Henneman and Mendell, 1981b). However, there is a complicating factor in the interpretation of these findings in masseter, as the Type IIB fibres are smaller than the Type I fibres (Eriksson, 1982). A large motoneuron, supplying many muscle fibres, may produce a smaller MacroRep measurement as the fibres supplied are only about half the diameter of the Type I fibres. Thus, a small MacroRep may well indicate a motor unit made up of a small number of large Type I fibres, or a large number of small Type IIB fibres (see Figure 2). Therefore, the finding that muscle units with small MacroReps are recruited first may mean that it is the muscle units supplied by large motoneurons that are recruited first. If so, recruitment of masseter motoneurons may not occur according to the size principle, whereas recruitment of muscle units does. There is some support for this possibility in the results of previous studies. In the limb muscles, there is a strong relationship between recruitment order and twitch contraction time, with the slower muscle units being recruited first (Rowlerson, 1990). This occurs because the small

muscle units consist of Type I fibres, which have slower twitch contraction times. This pattern has not been found in the jaw muscles (Goldberg and Derfler, 1977a; Yemm, 1977a), suggesting that the small motor units may be comprised of the smaller Type II fibres found in the masseter. Goldberg and Derfler (1977a) accepted spike amplitude as a measure of muscle fibre size and found that recruitment occurred in order of spike amplitude. However, as the Type II fibres are smaller than the Type I fibres in masseter (Eriksson, 1982), this may be interpreted as meaning that it is the large motoneurons, supplying the fast muscle units, which are recruited first. This finding may also have occurred because spike amplitude may not be an accurate indicator of muscle fibre size (Miles and Türker, 1986).

To interpret the findings that masseter muscle units are recruited in order of MacroRep size, and to draw any conclusions regarding the recruitment order of motoneurons, it is necessary to establish the relationship between MacroRep amplitude and motoneuron size in human masseter. Motoneuron size cannot be directly measured in humans, but is closely correlated to the axonal conduction velocity (Kernell and Zwaagstra, 1980), which can be inferred from the latency of H-reflex responses (Awiszus and Feistner, 1993).

The aim of this study was to investigate whether MacroRep amplitude, a measure of muscle unit size, can be used as an indicator of motoneuron size in masseter. This will be investigated by determining the relationship between MacroRep amplitude and H-reflex latency in masseter motor units.

7.2 *Materials and Methods*

Nineteen healthy volunteers (7 males, 12 females, age range 20 - 40 years) participated in the study.

7.2.1 H-reflex stimulating electrodes

The anode and cathode were incorporated into a U-shaped stainless steel frame, which has been described in Chapter 5.

7.2.2 Recording electrodes

Macro and SMU EMG were recorded as described in Chapter 5.

7.2.3 Protocol

To determine the maximum surface EMG activity in masseter, subjects were asked to bite as hard as possible three times. The surface EMG record was rectified and filtered (DC - 1Hz). The maximum value of surface EMG (in mV) for the three attempts was considered the maximum voluntary activity (MVA) in masseter.

The signal from the Macro EMG electrodes was observed on-line, triggered by the stimulus, to observe the presence or otherwise of an H-reflex in masseter. The Macro

EMG was shown in previous experiments (Chapter 5) to more clearly illustrate the H-reflex, and to allow better separation from the M-wave.

With the transmuscular stimulating frame in place, subjects gradually increased masseter activity until a motor unit action potential was observed on an oscilloscope. Action potential recognition on-line was achieved using the template-recognition program. The subjects were then asked to control the discharge of the masseter motor unit at a steady frequency with the assistance of audio feedback. The activity in masseter, as a percentage of the MVA, was measured from the rectified, smoothed surface EMG readings.

While the motor unit was firing steadily, the masseteric nerve was stimulated. A Digitimer Ltd. Isolated Stimulator (Model DS2) with a constant current device was used to generate the stimulus. Stimulus pulse width was 1 ms and the stimulus was applied randomly at a frequency of 0.2 - 0.5 Hz. The stimulus intensity and position were adjusted until an H-reflex could be observed in the Macro EMG. To determine that the response was in fact an H-reflex, the effect of increasing the masseter activity level was observed. Increasing the masseter activity level would increase the size of an H-reflex without changing the size of an M-wave. Once an H-reflex was observed in the Macro EMG, the stimulus position and intensity were fixed, and stimulation continued until the subject was no longer able to fire the unit regularly. The subject then rested for at least 10 minutes, after which the procedure was repeated, with a different motor unit. The results from motor units that could not be maintained for at least 200 stimuli were

discarded. Macro EMG and motor unit activity in masseter were recorded on videotape for off-line analysis.

7.2.4 Analysis

The shape of the motor unit action potential was recognised using the template-matching program. PSTHs of the firing of an identified motor unit were constructed from the recognition pulses of the spike program. To determine whether an H-reflex was present, CUSUM of the PSTH was calculated, along with significance lines (Davey et al. 1986). If the CUSUM crossed the significance limits, then an H-reflex was accepted as present.

The amplitude of the MacroRep was used as the indicator of muscle unit size, determined as described in Chapter 6.

The latency of the H-reflex in a motor unit was determined from the take-off point of the CUSUM. The time between the onset of the MacroRep and the trigger spike was used to estimate the intramuscular delay (Awiszus and Feistner, 1993). This was then subtracted from the latency measured from the CUSUM to determine the corrected H-reflex latency.

7.3   Results

H-reflex responses could not be elicited in three subjects. The results presented are from the remaining 16 subjects, from whom H-reflex responses were recorded in a total of 42 motor units. In one subject, H-reflexes were recorded in four different units. In eight subjects, H-reflexes were recorded in three units, and in seven subjects H-reflexes were present in two units. H-reflex responses were only recorded when activity was above 10% of the MVA. H-reflex responses were recorded at masseter activity levels up to 30% of MVA. Beyond this level it became difficult to consistently identify every firing of individual motor units.

For each subject, the MacroRep amplitudes were plotted against H-reflex latencies, as shown in Figure 16(a). It was inappropriate to pool raw results between subjects, as the H-reflex latencies would have been affected by the size and shape of the subjects' jaws, and by the placement of the electrodes. This explains why a variety of MacroRep amplitudes appear to occur for the same H-reflex latency. Thus the plots in Figure 16a can only show the relationship between corrected H-reflex latency and MacroRep amplitude in individual subjects. As can be seen, in every case except one (shown by a orange line), corrected H-reflex latency increased as MacroRep amplitude decreased. This occurred at low and high biting levels. To enable pooling of the results from different subjects, it was necessary to standardise the MacroRep amplitudes and H-reflex latencies to the mean for each subject. The mean and standard deviations of the H-reflex latency and MacroRep amplitude of each subject were calculated. Each score was then represented by its number of standard deviations (SD) above or below the
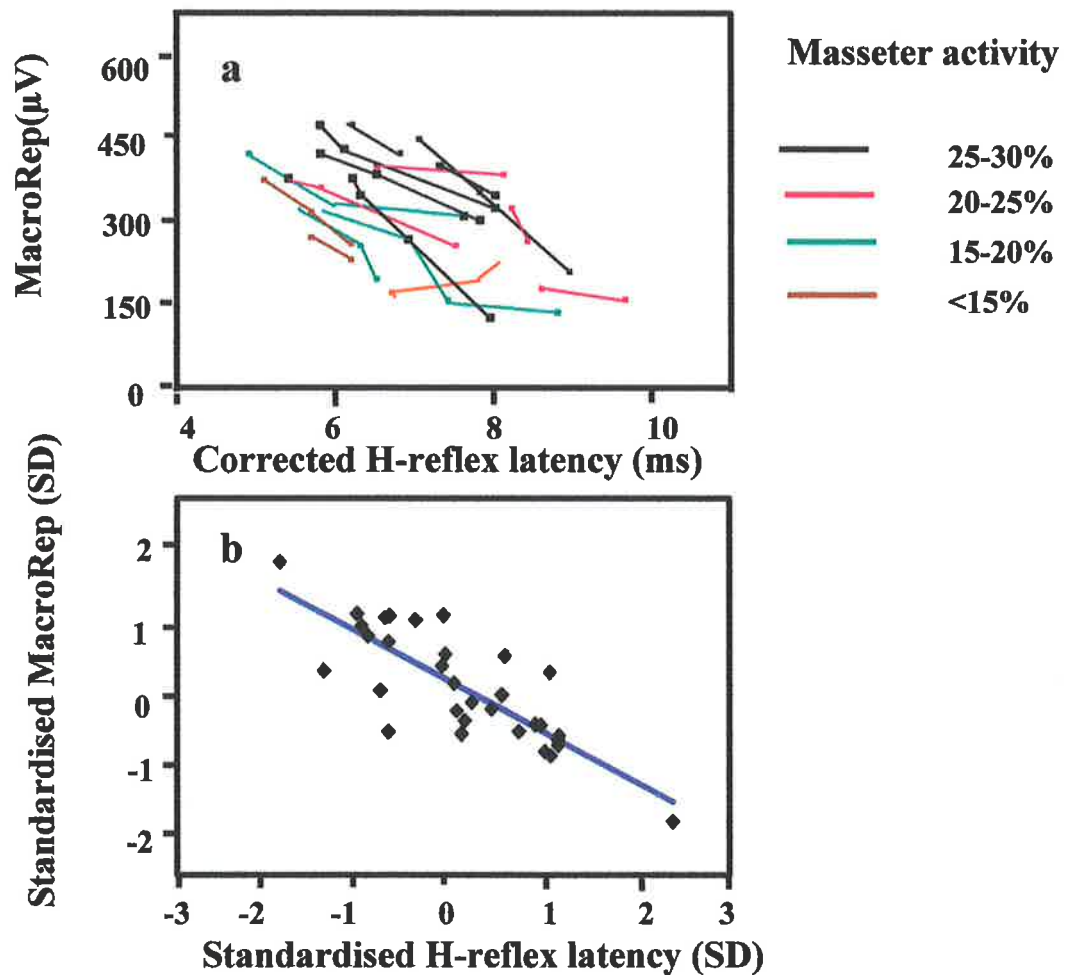
Figure 16. Relationships between H-reflex latency and MacroRep amplitude in 16 subjects.

(a) Corrected H-reflex latency and MacroRep amplitude are plotted in 16 subjects. In 15 subjects there was a decrease in MacroRep amplitude as H-reflex latency increased (solid lines). In one subject, Macrorep and H-reflex latency increased together (orange line: biting level <20%). The level of masseter activity, as a percentage of maximum surface EMG, is indicated by the colour of the lines. (b) H-reflex and MacroRep amplitudes were standardised to the mean for the subject, and represented as standard deviations (SD) above or below the mean. There was a significant negative correlation between the standarised scores for H-reflex latency and MacroRep amplitude: $r = .75$, $p < 0.001$.

mean score for the subject. The standardised scores were plotted, as shown in Figure 16(b). This enabled the relationship between H-reflex latencies and MacroRep amplitudes to be investigated in the pooled results. There was a significant negative correlation ($r = -.75$, $p < 0.001$) between the standardised scores for H-reflex latency and MacroRep amplitude.

7.4 Discussion

The H-reflex latency was used as an indicator of motoneuron size in this study, based on the established relationship between axonal conduction velocity and motoneuron size (Kernell and Zwaagstra, 1980; Henneman and Mendell, 1981b). The H-reflex latency is composed of afferent conduction time, synaptic delays and efferent conduction time. For units from the same subject, it is safe to assume that afferent conduction time and synaptic delay will be the same for each unit. Thus differences in H-reflex latency may be attributed to differences in motoneuron size (Awiszus and Feistner, 1993).

This study clearly showed that units with a shorter H-reflex latency have a larger MacroRep amplitude. This relationship would be expected if the larger motoneurons supply muscle units with a larger cross-sectional area. It appears that the smaller size of the Type II muscle fibres in masseter does not reduce the MacroRep to a great enough extent to disrupt the normal relationship between MacroRep size and motoneuron size. From low-threshold units, Stålberg et al. (1986) estimated that small units would contain 100 muscle fibres or less. Comparing this to Carlsöö's (1958) mean value from all units of 640 fibres/unit, suggests a large range in the number of muscle fibres per

unit. Thus the smallness of the Type II fibres must be counterbalanced by the large number of muscle fibres in the large muscle units, allowing the muscle unit size (measured by MacroRep) to be an appropriate indicator of motoneuron size in masseter.

The relationship between MacroRep and H-reflex latency was only demonstrated for motor units recruited between 10% and 30% of MVA. Although individual motor units could be recognised above 30% of MVA, it was not possible to identify every firing of individual motor units. Below 10%, an H-reflex could not be elicited, and hence those motor units that are recruited at low activity levels could not be included in the study. By necessity the relationship between MacroRep amplitude and H-reflex latency could only be investigated in motor units that demonstrated an H-reflex could be investigated.

H-reflexes were difficult to elicit, indeed in three of subjects no H-reflexes could be elicited, despite many attempts with different electrode positions, masseter activity levels and stimulus intensities. Even in subjects demonstrating a clear H-reflex in the Macro EMG, only a limited number of motor units demonstrate the H-reflex. This situation is analogous to the findings of Miles et al. (1995) who found that 35% of masseter motor units did not demonstrate a short-latency stretch reflex. The limited prevalence of H-reflexes in masseter motor units prevents H-reflex latency being useful as a tool for indicating motoneuron size in most motor units, and also limits to the conclusions which can be drawn from the current study. Thus, the relationship between H-reflex latency was only established for motor units that actually demonstrated an H-reflex. However, as this relationship was established across a wide range of MacroRep sizes and H-reflex

latencies, and in 15 out of 16 subjects, extrapolations to other motor units in deep anterior masseter can be made with reasonable confidence.

In this study, H-reflexes and MacroRep were only recorded for motor units in deep anterior masseter. As there are at least three compartments in masseter; deep anterior, deep posterior and superficial (van Eijden et al. 1993; Blanksma et al. 1997), these conclusions may not necessarily be generalised to the entire motoneuron pool for masseter.

Schmied et al. (1997) found only weak correlations between H-reflex latency and the representation of the motor unit in the surface EMG, in the wrist extensor muscles of humans. This contrary finding may be because the "SurfaceReps" in that study were determined by the averaging of the surface EMG record rather than the Macro EMG. The MacroRep determined from surface electrodes is largely dependent on the depth of the unit investigated and cannot be regarded as a reliable measurement of motor unit size (Awiszus and Feistner, 1993). Whether the MacroRep determined from surface electrodes is a reliable measure of motor unit size is debateable (Awiszus and Feistner, 1993, Roeleved et al., 1998).

7.5   Summary and Implications

In Chapter 6, it was shown that the recruitment of masseter muscle units occured in order of size, when the muscle unit size was measured by the amplitude of the

MacroRep. The relationship between MacroRep size and H-reflex latency has now been demonstrated. If H-reflex latency is accepted as a measure of motoneuron size, then a relationship has been established between muscle unit size and motoneuron size in human masseter. Therefore, the MacroRep amplitude of muscle units in masseter gives a valid indication of motoneuron size. Applying this finding to the results of the previous chapter allows the conclusion to be drawn that masseter motoneurons are recruited according to the size principle during slow voluntary isometric contractions.

During this study, and during the study described in Chapter 5, the difficulty of eliciting H-reflexes in masseter was noted. In some subjects, it was not possible to elicit H-reflexes at all, and even in those subjects in whom strong H-reflexes were observed in the Macro EMG, H-reflexes were only noted in a small percentage of motor units. The question remains as to why some motor units demonstrate the H-reflex and others do not. That the H-reflex results from input from the Ia afferents is known, but what it is about the distribution of the Ia afferent input that results in some motor units demonstrating the H-reflex while others do not, has not yet been established.

From the results of Chapters 6 and 7 it is possible to conclude that recruitment of masseter motoneurons usually occurs according to the size principle, and that the amplitude of the MacroRep provides a useful indicator of motoneuron size. Using this information, and the methodology developed in Chapter 5, the recruitment of masseter motoneurons into the H-reflex will be investigated in the next study, in order to

determine the effectiveness of the Ia afferent input onto masseter motoneurons of different size.

Chapter 8. Effectiveness of Muscle Spindle Input onto Masseter Motoneurons

8.1 Introduction

H-reflexes are small and can be difficult to elicit in the masseter in humans (Godaux and Desmedt, 1975) and animals (Türker, 1978) even though these muscles contain large numbers of muscle spindles (Rowlerson, 1990). H-reflexes are also difficult to elicit in the small muscles of the hand (Schieppati, 1987) and it has been suggested that this may be due to a skewed distribution of Ia afferents onto the large motoneurons (Mazzocchio et al. 1995). If Ia afferent input is distributed in this way, it would be difficult to evoke H-reflexes, as the large motor axons, which would be conveying the H-reflexes, are the first to be blocked by antidromic impulses due to the stimulus. Additionally, large voluntary contractions would be needed during experiments to elicit H-reflexes, to recruit the larger motoneurons.

A more effective input from muscle spindle afferents onto the large masseter motoneurons may explain the difficulty of eliciting H-reflexes in masseter. This type of distribution may also have ramifications for the role of the muscle spindles in the control of jaw movements during speech and mastication, and for the maintenance of jaw posture. Therefore, the aim of this study is to determine the effectiveness of the Ia

afferent input onto simultaneously active large and small motoneurons, by comparing H-reflex responses in the motor units.

8.2 Materials and Methods

Fifteen healthy volunteers (9 females, 6 males, age range 21 - 38 years) participated in the experiments.

8.2.1 Stimulation of the masseteric nerve

The transmuscular stimulating frame described in Chapter 5 was used to stimulate the masseteric nerve.

8.2.2 Recording electrodes

Surface EMG, Macro EMG and SMU recording electrodes were used, as described in Chapter 5. However, instead of a single bipolar SMU electrode, two SMU electrodes were used. Each inserted electrode consisted of five wires; two bipolar SMU electrodes and the Macro electrode. The tips of both SMU electrodes were positioned against the Macro electrode, with the tips of the electrode pairs separated by 2-3 mm, as shown in Figure 17. This arrangement allowed recording from two different motor units simultaneously, without the possibility of superimposition of the action potentials. Occasionally, two motor units were recorded from one or both SMU electrodes, allowing 3-4 motor units to be followed simultaneously.
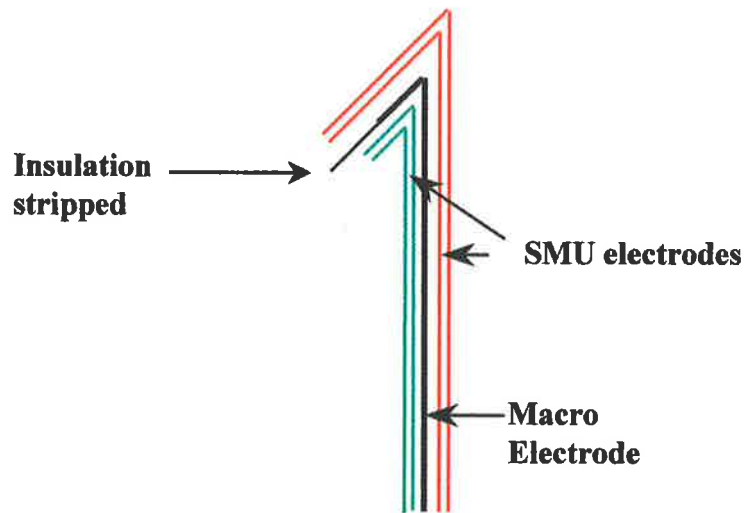
Figure 17. Electrode for Recording from Two Motor Units and Macro EMG.

The recording electrode consisted of a Macro electrode (black) and two pairs of SMU electrodes (red and green) . The SMU electrodes were positioned against the area of the Macro electrode from which the insulation had been removed . The SMU electrodes were separated from each other by 2 - 3mm.

8.2.3 Protocol

To determine the maximum surface EMG activity of left masseter, the subjects were asked to bite as hard as possible three times. The surface EMG signal was rectified and filtered (DC-1Hz) and the activity recorded in mV. The maximum level of surface EMG of the three attempts was considered the maximum voluntary activity (MVA) of the left masseter.

With the subject contracting their left masseter to 5%, 10%, 15% or 20% of MVA, (using the rectified, smoothed surface EMG of the masseter given as visual feedback), the stimulus was increased until an H-reflex was noticeable in the Macro EMG. The intensity and position of the stimulus was then adjusted until an H-reflex was present with minimum M-wave. H-reflex was differentiated from M-wave or F-wave as described in Chapter 5. For each subject, H-reflexes were investigated at the 4 different MVA levels. At contraction levels above 25%, too many motor units were recruited to enable individual motor units to be accurately and consistently identified, without superimposition of other motor units. To ensure that fatigue of the subject did not affect the results, the order of contraction levels was randomised.

The template-matching program was used to identify the firing of the active motor units at the chosen contraction level. Subjects often needed to slightly increase or decrease the masseter activity level, in order to allow regular firing of a clearly identified motor

unit. The actual activity level in masseter, as a percentage of MVA, was recorded. The subject received audio feedback of the firing of one of the motor units; this ensured that the contraction level of the masseter was kept reasonably constant (Türker and Miles, 1989).

With audio feedback, subjects were able to voluntarily regulate the firing rate of a particular motor unit so that it ran at a steady frequency of 10 - 18 Hz. While the motor unit was firing steadily, the masseteric nerve was stimulated as described in Chapter 5. Stimulus intensities were below the M-response threshold in the motor units studied, ensuring that the responses obtained in the motor units were H-reflexes and not F waves (Hugon, 1973). Each motor unit was run voluntarily by the subject for at least 200 stimuli. (about 10 -14 minutes). For each period of recording ("run") the stimulus intensity and position and the masseter activity level were stable. The Macro EMG was continually averaged, triggered by the firing of the feedback motor unit. The shape and amplitude of the resulting MacroRep was constantly monitored to ensure that the same motor unit was being followed. During each run, the firing patterns of 2-4 motor units was recorded. To avoid fatigue effects, the subjects rested for not less than 10 minutes at the completion of each run, after which the procedure was repeated with different motor units at a different masseter activity level.

After some runs, the subject was asked to rest for a brief period with the stimulator in place. After a short rest (2-3 minutes) the stimulus was applied at the same intensity as before, and the subject attempted to recruit the same motor units at a different

contraction level. The on-line MacroRep and the template-matching program were used to determine if the subject had been able to recruit the same motor units. Examining the same motor units at different contraction levels, with constant stimulus position and intensity, enabled the effect of contraction level on H-reflexes in motor units to be determined.

8.2.4 Analysis

Muscle unit size (MacroRep): The size of the muscle units was determined from the amplitude of the MacroRep, as described in Chapter 7.

H-reflex in Macro EMG: The Macro EMG was averaged using the stimulus as a trigger, to determine the onset latency of the masseteric H-reflex responses.

H-reflex in motor units: The motor unit potentials were filtered to single out the shape and amplitude of the units of interest and to depress the amplitudes of any other units.

PSTHs were produced for the identified motor units. CUSUM and significance curves were calculated to determine the presence of a reflex response and the latency of onset. The H-reflex size was determined from the PSRR. As PSRR will depend on stimulus intensity and position, PSRR values were only compared for motor units within a run. The incidence of H-reflexes in large and small motor units was compared, and a sign test

was used to determine if the difference was significant. A p value of <0.05 was accepted as significant.

As motor unit firing frequency may affect the size of the reflex response (Jones and Bawa, 1995), the firing frequencies of the units were determined using the off-line (POSTPROC) facility in SPS-8701. A general linear model for unbalanced data was used to investigate whether there was a difference in firing frequencies of the units with and without an H-reflex.

When motor units were recorded simultaneously in one SMU electrode during the same time interval (12 runs) it was necessary to ensure that an H-reflex in a smaller unit was not missed due to simultaneous firing of a larger unit. Therefore, the firing intervals of all motor units were inspected. A larger firing interval in a smaller unit, when occurring in relation to the stimulus and with the firing of a larger unit, may indicate superimposition. This was a very rare event (3 occasions) and when it was observed, the PSTHs of the smaller units were adjusted to allow for the possibility of "missing" occurrences. This correction did not result in H-reflexes in any motor units in which they had previously not been present.

H-reflex latency was determined from the CUSUM, and was corrected using the MacroRep as described in Chapter 5.

To determine whether cutaneous stimulation affected the reflex response of the motor units, in two subjects (4 runs) the cathode was moved anteriorly, so that the stimulus was applied within the distribution of the maxillary branch of the trigeminal nerve but was not over the masseteric nerve. The stimulation and recording procedures were repeated with the stimulus in this position, using the same stimulus current as was applied over the masseteric nerve.

8.3 Results.

H-reflexes could not be elicited in three subjects, despite many attempts with different stimulus intensities and positions. In the 12 subjects in whom H-reflexes could be elicited, an H-reflex was identified in the Macro EMG in 23 runs; within each run, 2-4 motor units were identified. The mean latency of masseter H-reflexes recorded in the Macro EMG was 6.4 ms (range 5.5 - 7.3).

Table 4 summarises the data from the 23 runs in which H-reflexes were elicited in motor units. Significant H-reflex responses were recorded in 27 out of a total of 66 voluntarily recruited motor units. In each run, H-reflexes were present in 1 or 2 motor units and not present in 1, 2 or 3 units. The amplitudes of the MacroReps for each of the motor units identified within a run were normalised to the size of the largest motor unit in the run, which was thus assigned a size of 100%. As can be seen from Table 4, the units that demonstrated an H-reflex were usually the larger units in a run. From the 23 successful

Table 4. H-reflex Responses in Motor Units.

The H-reflex responses of 67 motor units (MUs) voluntarily recruited during 23 runs is shown. H-reflexes were present in 27 of the motor units. Motor unit size was expressed as a percentage of the MacroRep of the largest motor unit in each run. In 21 out of the 23 runs, H-reflexes were present in the larger motor units. The runs where this was not the case are highlighted. H-reflex latency (ms: corrected with MacroRep) and size (PSRR) for each motor unit demonstrating an H-reflex is shown, as well as the firing frequency (FF) of each motor unit. In each run, the H-reflex was present in the Macro EMG, and the latency of the response (in ms) is shown. Masseter activity of at least 10% of MVA was required to elicit H-reflexes in masseter

Run	H-reflex latency in Macro (ms)	Masseter Activity %MVA	Motor Units with H-reflex								Motor Units with no H-reflex					
			MU Size (%)	H-reflex Size (PSRR)	FF (Hz)	Lat (ms)	MU Size (%)	H-reflex Size (PSRR)	FF (Hz)	Lat (ms)	MU Size (%)	FF (Hz)	MU Size (%)	FF (Hz)	MU size (%)	FF (Hz)
1	5.8	10	100	.21	17.1	6.2										
2	6.4	10	100	.11	14.4	6.4					66	17.1	56	16.9		
3	6.2	10	100	.14	14.1	6.7					65	14.4	45	13.8		
4	7.1	10	100	.16	14.2	6.1					50	14.1	50	14.9		
5	5.9	12	100	.13	13.0	6.2	75	0.12	14.2	8.7	70	14.2	60	13.8	30	16.7
6	7.2	12	100	.19	13.0	6.8					38	14.5	31	13.9		
7	6.8	12	100	.12	12.1	6.4					62	13.5				
8	6.4	14	100	.13	13.2	5.4					42	12.3				
9	6.0	14	100	.21	14.5	7.2					67	13.9				
10	5.4	14	100	.18	14.2	6.8					65	15.4				
11	5.9	15	100	.17	14.1	7.4	83	0.11	12.3	9.1	67	17.5	45	15.9		
12	7.3	15	100	.13	19.6	8.1					34	16.1				
13	6.7	15	100	.30	17.8	5.9					55	16.1				
14	6.5	16	100	.21	12.8	6.2					65	13.9				
15	5.9	17	100	.19	15.5	6.8	90	.11	16.9	8.1	41	16.4				
16	5.8	17	100	.14	18.5	7.5					71	17.3	20	12.5		
17	7.1	17	100	.12	15.2	7.7					43	19.6	25	16.1	15	15.4
18	6.7	18	48	.20	16.5	7.3					52	14.5				
19	6.6	18	100	.14	14.1	7.6					100	15.9	36	17.4		
20	6.4	19	77	.14	11.1	6.1					48	14.5				
21	6.8	19	100	.16	15.2	5.9					100	12.3				
22	7.2	19	100	.21	12.7	8.1	86	.11	16.7	7.8	47	13.3				
23	5.9	20	100	.29	16.5	7.5					40	15.2				
											74	18.9	43	17.4	27	15.3

runs shown in Table 4, 9 runs had 2 voluntarily activated units, one of which demonstrated the H-reflex and one which did not. Of these 9 pairs, in 8 cases the larger unit (size 100%) was the one demonstrating the H-reflex (Table 4, runs 6-9, 12-14, 20,21). In 14 runs, 3 - 4 units were identified, some with and some without the H-reflex present. In 13 of these runs the unit/s with the largest MacroReps were those that demonstrated an H-reflex (Table 4, runs 1-5,10,11,15-19,22,23). In total, in 21 runs out of 23, the H-reflex occurred in the larger units (sign test, $p < 0.001$). In four runs, H-reflexes were recorded in two motor units simultaneously (runs 5, 11, 15, 22). In each case, the larger unit had a stronger H-reflex, as indicated by the PSRR. However, the small number of such incidences precluded statistical analysis.

Table 4 also shows the firing frequencies (FF) of the motor units. There was no significant difference between the firing rates of the units with and without H-reflexes (unbalanced ANOVA, $F = .12$, $p = .73$). The mean raw latency for the H-reflex in the motor units was 7.5 ms (range 5.9 - 10.2 ms). When corrected for the intramuscular delay, the mean latency was 7.0 ms (range 5.4 - 9.1 ms).

On nine occasions it was possible to identify the same motor unit at different masseter activity levels, with the same stimulus position and intensity. This enabled the effect of contraction level on H-reflexes in the same motor unit to be determined. These results are summarised in Table 5. In this Table, the sizes of all the motor units recruited by a subject in 2-3 runs were normalised to the size of the largest motor unit. The motor unit sizes were then ranked. Table 5 shows that at each contraction level, it was the

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Series	Motor units active at different masseter activity levels		
	18 - 22 % MVA	13 - 17% MVA	<12 % MVA
1		① ❷	❷ ❸
2		① ❷	❷
3		① ② ❸ ❹	❸ ❹
4	① ❷ ❸ ❹	① ❹	
5	① ❷ ❸	① ❷	
6	① ❷ ❸ ❹		❷ ❸
7	① ❷		❷
8	① ② ❹	② ③ ❹	❹ ❺
9	① ❷ ❸	① ❷ ❸	

Table 5. Effect of Masseter Activity Level on H-reflexes in Motor Units

On 9 occasions it was possible for the subjects to run the same motor units at different contraction levels. A series consists of 2 - 3 runs performed by the same subject, with the same stimulus intensity and position. For each series, all of the motor units recruited in the runs of one subject are described in rank order according to their size. The units which displayed an H-reflex are shown in an open circle; those without an H-reflex are shown in a filled circle. Thus it can be seen that in Series 1, there were 2 runs, one with the subject contracting masseter at 13 - 17 % MVA and one with MVA of less than 12 %. When the subject was contracting masseter at 13 - 17 % MVA there were 2 motor units running. The larger of these (①) displayed an H-reflex. The smaller (❷) did not. When the subject decreased the contraction level to <12 % MVA, motor unit ❷ was still recruited and still did not demonstrate an H-reflex; neither did a smaller unit ❸, which could only be recognised at this contraction level. The larger motor unit ① was not recruited at the lower contraction level. In every series, small motor units did not demonstrate an H-reflex even at higher contraction levels. The data from Series 8 are illustrated in Figure 18.

larger units that demonstrated an H-reflex. Smaller motor units did not demonstrate an H-reflex even when they were identified at higher masseter activity levels. Figure 18 shows the results from one subject. When the masseter activity level was 19%, three motor units were followed. The larger two motor units demonstrated an H-reflex and the smaller motor unit did not. When the activity level was decreased to 15%, two large motor units were followed; one of which was the same as had been followed previously (indicated by an arrow). When masseter activity was decreased to 5%, two small motor units were followed. There was no H-reflex in the Macro EMG, nor in either of the units, at this activity level. This Figure clearly shows that the larger units demonstrated an H-reflex irrespective of the contraction level, and that at higher contraction levels the smaller motor units still did not demonstrate an H-reflex. The same pattern was observed on the 8 other occasions where the same motor units were identified at different activity levels (Table 5).

In seven runs (from 6 subjects), motor units that were not voluntarily recruited fired frequently at a latency of 4.0 - 5.5 ms. As they fired only directly in relation to the stimulus, these were termed "stimulus-recruited" (SR) units. To determine the size of the SR units, the Macro EMG was averaged, triggered by the stimulus, on consecutive occasions in which the unit fired. The Macro EMG was then recorded on a similar number of occasions in which the unit did not fire. The difference in the amplitude between the two occasions corresponds to the size of the MacroRep of the stimulus-recruited unit. Figure 19 shows the Macro EMG averaged with and without the SR unit firing, in one subject. The MacroRep of the SR unit is shown with double-headed
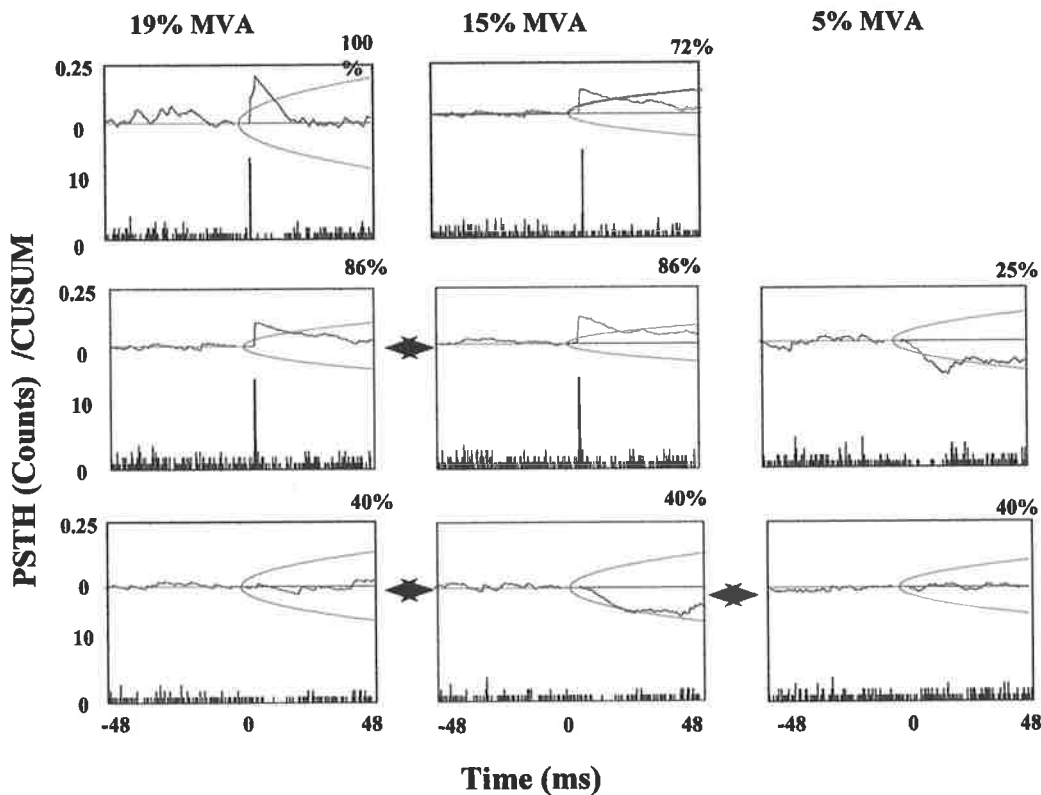
Figure 18. H-reflex responses in size different motor units identified in 3 runs in one subject.

The subject was biting at different levels of maximum voluntary activity (MVA) in each run, but the stimulus intensity and position were the same. Each chart shows the PSTH of the motor unit with the CUSUM and significance curves. An H-reflex was accepted as being present in the CUSUM record crossed the significance curves. The size of the MacroRep of the motor units is expressed as a percentage of the size of the largest motor unit in the 3 runs. At 5% MVA two small units were identified (MacroRep 25% and 40%). No H-reflex was present in either motor unit. At 15% MVA three motor units were identified, two larger units and one which had been present at 5% MVA (indicated by arrow). An H-reflex was present in the two larger units (MacroRep 72% and 86%) but was not present in the smaller unit. At 19% MVA three units were identified. An H-reflex was present in the largest unit (100%) and the other large unit (86%: also present at 15% MVA) but not in the smallest unit (40%). In two units (smallest at 15 % and larger at 5%) there was a decrease in motor unit firing after the stimulus.

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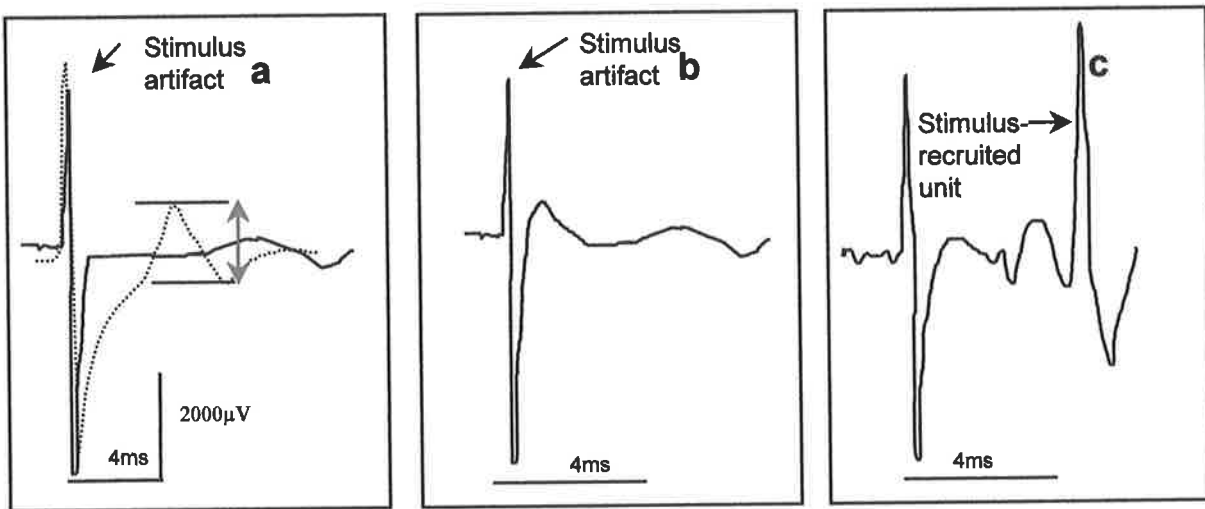
Figure 19. Stimulus-recruited Unit Shown in the Macro EMG and in the SMU Electrode Output.

(a) The Macro EMG was averaged (n=7) when the stimulus-recruited unit was firing (dotted line) and not firing (solid line). The amplitude of the representation in the Macro EMG of the stimulus-recruited unit (MacroRep) is shown by the double-headed arrow. The oscilloscope trace of the SMU electrode output is shown when the stimulus-recruited unit was not firing (b) and firing (c) at a latency of 4.1 ms.

arrows. Figure 19 also shows the motor unit electrode output when the SR unit was not firing (b) and firing (c). The SR units were very large compared to the voluntarily recruited units, and they fired in response to many, but not all, stimuli. They were only observed at masseter activity levels that were at least 15% of MVA.

Table 6 shows the runs in which SR units were observed. In all cases, the stimulus-recruited units were larger than the voluntarily recruited units, and their reflex latencies were always shorter. The H-reflexes were stronger in the stimulus-recruited units than in the voluntarily-recruited units, as shown by the larger PSRR values in the Table.

Figure 20 shows the CUSUM of the PSTH of a stimulus-recruited unit. The unit fired 108 times in response to 220 stimuli at a masseter activity level of 15%, producing a very strong H-reflex response at a latency of 4.3 ms. At the same time as the stimulus-recruited unit was observed, two other units were active during the voluntary activity level (Figure 20 (b) and (c)). The larger of these two units had an H-reflex (b), the smaller did not (c). The voluntarily recruited unit had a smaller H-reflex than the SR unit, as indicated by the PSRR.

In two subjects, it was possible to voluntarily recruit the stimulus-recruited unit, by increasing the activity level in masseter. Figure 21 shows the responses of one such unit, when it was stimulus-recruited, at a activity level of 15%, and when it was running voluntarily, at an activity level of 40%. The stimulus intensity and position were kept the same at the two activity levels. Of interest is the fact that the H-reflex response

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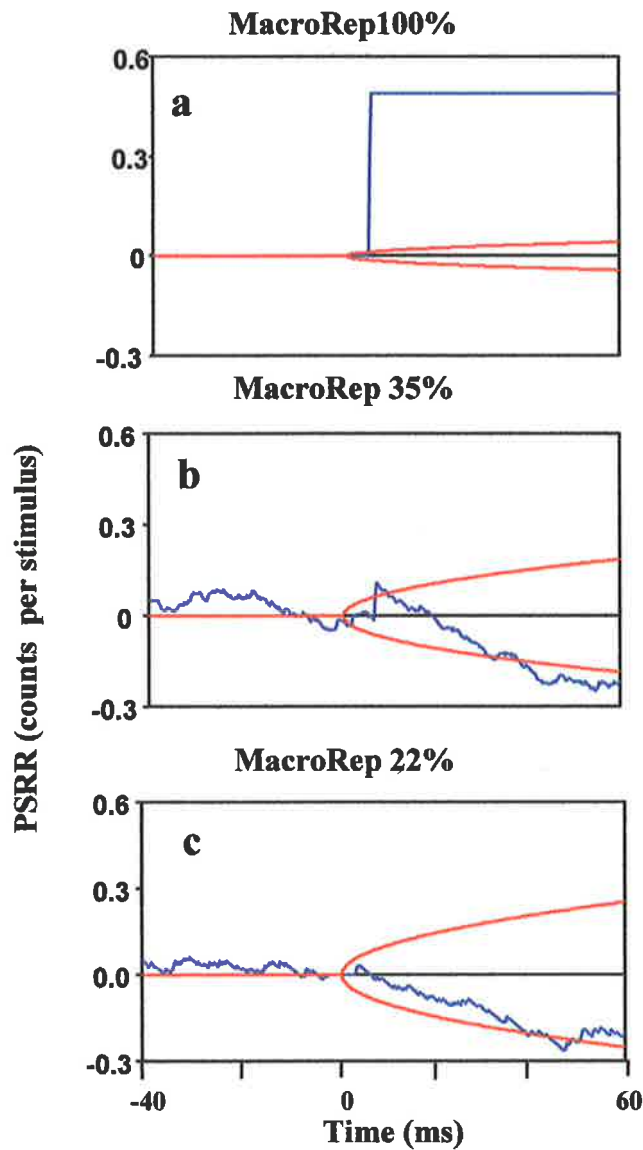
Figure 20. H-reflex in Stimulus-recruited and Voluntarily-recruited Motor Units.

CUSUM and significance curves for a stimulus-recruited unit (a) and two voluntarily recruited units (b,c) recorded during a single run in one subject. The amplitude of the MacroReps were normalised to the the MacroRep of the stimulus-recruited unit. An H-reflex was present in the stimulus-recruited unit and in the larger of the two voluntarily recruited units. The data illustrated is from run 1, Table 5.

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Run	Activity level (%MVA)	Motor Units with H-reflex						Units with no H-reflex		
		SR Unit			VR Unit					
		Size (%)	Latency (ms)	PSRR	Size (%)	Latency (ms)	PSRR	% size	% size	%size
1	15	100	4.3	.52	35	7.7	.12	22		
2	15	100	4.6	.61				33		
3	15	100	5.1	.46	43	6.7	.20	25		
4	18	100	4.2	.54	35	7.2	.19	29	25	
5	18	100	5.4	.58				35	25	22
6	20	100	4.0	.64	76	7.4	.15	30		
7	20	100	5.1	.48				30	20	

Table 6. Stimulus-recruited and Voluntarily-recruited Motor Units.

In seven runs, stimulus-recruited (SR) and voluntarily-recruited (VR) motor units could be observed at the same time. The amplitude of the MacroReps of all units in each individual run were normalised to the amplitude of the stimulus-recruited (SR) motor unit. In every case, the SR unit was larger than the VR units. H-reflexes were demonstrated in the SR units and in some of the larger VR motor units. No H-reflexes were demonstrated in the small motor units. In every case the H-reflex was stronger in the SR units (PSRR was larger) and the corrected H-reflex latency was less than in the VR motor units. The contraction level in masseter was at least 15% MVC in each run.
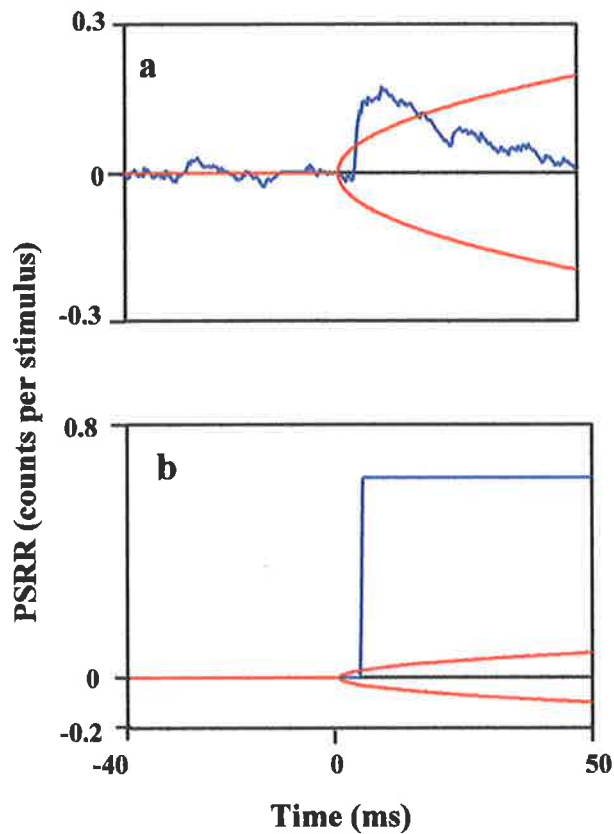
Figure 21. H-reflex Response when the Same Motor Unit is Running Voluntarily and is Stimulus-recruited.

The same motor unit was recorded as a voluntarily-recruited unit (a) and as a stimulus-recruited unit (b). Stimulus intensity and position were the same in each case. The masseter activity level was about 40% of MVA when the unit was recruited voluntarily and 15% when it was stimulus-recruited. The strength of the H-reflex response (PSRR) was less when the contraction level was higher. Note the difference in scales on the ordinate axes of the two graphs.

decreased in size, from a PSRR of 0.61 when the masseter was active at an activity level of 15% MVA, to a PSRR of 0.18 when the activity level increased to 40% MVA. The results for this motor unit and the other stimulus-recruited unit that was voluntarily recruited are shown in Table 7. Although in some cases it was possible to voluntarily recruit other stimulus-recruited motor units by increasing masseter activity levels, the recruitment of many other additional motor units made accurate identification unreliable in these cases.

In order to differentiate a cutaneous response to the stimulus from an H-reflex, the stimulus was moved anteriorly in two subjects, after the completion of a successful run. Figure 22 shows the responses to the masseteric nerve and cutaneous stimulation during one run in one subject. When the stimulus was applied over the masseteric nerve, two motor units were followed. The larger of these units (MacroRep 100%) demonstrated an H-reflex, while the smaller one (MacroRep 42%) did not. When the stimulus was applied anteriorly (cutaneous stimulation), neither unit displayed an H-reflex. The same result was found with the 3 other runs where the stimulus was moved, ie, units that had demonstrated an H-reflex with masseteric stimulation did not do so with cutaneous stimulation. There was, however, a small decrease in the CUSUM of the PSTH, indicating that the cutaneous stimulation may induce inhibition. The inhibitory cutaneous response may explain the longer latency inhibition seen in some cases where an H-reflex was not present (see Figure 18).
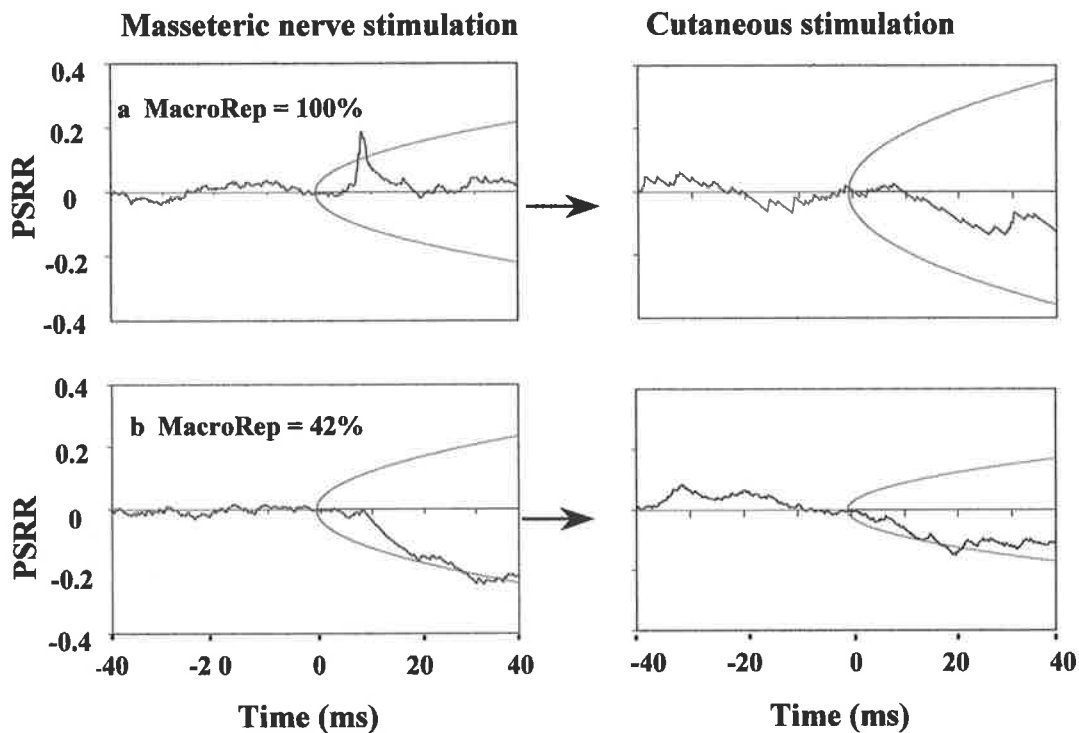
Figure 22. Effects of Masseteric Nerve and Cutaneous Stimulation.

Stimulation applied to the masseteric nerve resulted in an H-reflex in a large motor

unit (a: MacroRep 100%) but not in a smaller unit (b: MacroRep 42%). When the

stimulus was moved anteriorly, within the distribution of the maxillary nerve but not

over the masseteric nerve(Cutaneous stimulation), neither motor unit demonstrated an

H-reflex.

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Masseter activity level	H-reflex size (PSRR)	H-reflex latency (ms)
Unit 1		
15%	.61	4.6
40%	.18	4.6
Unit 2		
20%	.48	5.1
45%	.11	5.1

Table 7. H-reflexes in Stimulus-recruited Motor Units When Biting Level is Increased.

H-reflex size (PSRR) and corrected latency (ms) are shown for two stimulus-recruited units which could be voluntarily recruited by increasing the masseter activity level. When masseter activity level increased, H-reflex size decreased, but H-reflex latency stayed the same.

8.4 Discussion

8.4.1 H-reflexes in Masseter Motor Units

Twelve subjects demonstrated masseter H-reflexes. Even when demonstrated in the Macro EMG, H-reflexes were not present in many of the motor units that were active. During the 23 successful runs, H-reflexes were elicited in only 27 (40%) of the 66 motor units observed. Similar findings have been described in stretch reflex responses in masseter: Miles et al. (1995) found that in 35% of motor units there was no short-latency stretch reflex response. In this study, in nearly all cases it was the larger motor units which demonstrated the H-reflex. Miles et al (1995) could not demonstrate a relationship between motor unit size (as indicated by discharge frequency) and stretch reflex size. However, only low-threshold units were investigated, so there may have been an insufficient range of motor unit sizes to demonstrate a relationship. Additionally, masseter motor units can change their firing pattern during prolonged contractions (Nordstrom and Miles, 1991a), making this an inappropriate way of determining motor unit size during the extended periods of contraction used in their experimental procedure.

Using the MacroRep as an indicator of motor unit size, the results of the current study clearly showed that the larger motor units displayed the H-reflex more often than the small motor units. CUSUM was used to detect the presence of H-reflexes in large and small motor units. Due to differences in the after-hyperpolarisation in large and small motoneurons, CUSUM may underestimate the size of the EPSP in small motoneurons,

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and overestimate the size of the response in large motoneurons (Burke, 1981). However, it is unlikely that this effect would be sufficient to produce the findings in this study: that H-reflexes were totally absent in small motoneurons.

The presence of H-reflexes in larger motor units can be explained by a preferential distribution of Ia afferent input onto the large motoneurons. Alternatively, the monosynaptic Ia input onto the small motoneurons could be pre-synaptically inhibited. The relative merits of these arguments are discussed below.

8.4.1.1   PSI prevents H-reflex in small units

Although some authors (Desmedt and Godaux, 1980) have stated that pre-synaptic inhibition (PSI) of Ia afferents is not present in the masticatory system, modulation of jaw jerk reflexes to support stability of the mandible has been demonstrated by Lobbezoo et al. (1993b), indicating that PSI is present. Morphological evidence for the basis of PSI in rats has been established by Luo and Dessem (1999). The source of PSI of masseter Ia afferents has not been established, but it could result from the temporomandibular joints, cutaneous afferents (Goldberg and Nakamura, 1977b), the lingual nerve (Goldberg, 1972) or the periodontal mechanoreceptors, all of which would have been activated during this experimental paradigm, although Lobbezoo et al. (1993b) did not consider that the periodontal mechanoreceptors were a likely source. The reduction in H-reflex size associated with the high levels of masseter activity needed to voluntarily recruit stimulus-recruited units (see Figure 21) may be explained by PSI of

the Ia afferent input. However, with only two examples of this phenomenon, more investigation is required before conclusions can be drawn. If PSI of Ia afferent input was responsible for the limited H-reflexes observed in motor units in this study, such PSI would need to be skewed in favour of the small motoneurons. However, in animal limb studies, PSI has been shown to be evenly distributed across the motoneuron pool (Zengel et al. 1983) and the same pattern has been demonstrated in humans (Desmedt and Godaux, 1980). However, Schmeid et al (1997) found that in the wrist extensors presynaptic inhibition can affect the Type F motor units more, so that they become more responsive. Whether there are other examples of differential distribution to large and small motoneurons remains to be seen. .

8.4.1.2   Ia input favours large motoneurons

There are opposing findings concerning Ia distribution onto motoneurons in human subjects. Awiszus and Feistner (1993) and Schmied et al. (1997) found that monosynaptic Ia EPSP size was greater in small motoneurons, and concluded that Ia afferent input supported the size principle in the soleus and wrist muscles respectively. Both of these studies relied on the Ia EPSP size, inferred from the PSTH, as an indicator of Ia afferent input. However, the amplitude of a synaptic potential may not be an appropriate indicator of the synaptic input because of its dependence on motoneuron size (Heckman and Binder, 1990). Therefore, small reversals in Ia input may be insufficient to overcome the effects of motoneuron size. Ashby et al. (1987) compared H-reflex responses in large and small muscle units in tibialis anterior (as indicated by

MacroRep amplitude) and found that synaptic efficacy of muscle afferents was equal in large and small muscle units.

In contrast, two previous studies support the findings of the present study. Semmler and Türker (1994) found that the H-reflex was larger in higher recruitment-threshold motor units in human tibialis anterior. Mazzocchio et al. (1995) demonstrated an uneven distribution of Ia afferents within the motoneuron pool of the hand, with input being ineffective on small, low threshold motoneurons. They concluded that there was a skewed distribution of Ia excitation to the motoneuron pool, favouring the discharge of large motoneurons over the small ones.

The findings of the current study clearly show that the larger motor units, as indicated by the amplitude of the MacroRep, were preferentially recruited in the H-reflex. Whatever the masseter activity level used by the subject, the larger units demonstrated a larger H-reflex. At low bite levels, an H-reflex was not apparent on many occasions because only small motor units had been recruited. Previous authors have noted that strong contractions in masseter are required in order to elicit an H-reflex (Godaux and Desmedt, 1975; Cruccu, 1989a; Macaluso and de Laat, 1995a), and it has been assumed that voluntary contraction is necessary to increase the excitability of the motoneuron pool. However, the results of this study suggest that voluntary contraction is needed in order to recruit the larger motoneurons, so that they may then participate in the H-reflex. This argument is strengthened by the fact that even at high bite levels, smaller motor units did not demonstrate an H-reflex. At higher bite levels, larger motoneurons

were recruited, and these demonstrated an H-reflex. However, once present in a motor unit, the H-reflex size did not change at higher bite levels (Figure 18), similar to the findings of Ashby et al. (1986) in human tibialis anterior and Miles et al (1989) in the human soleus.

H-reflexes in masseter have previously been found to respond differently to vibration than H-reflexes in the limb muscles. In the limbs, H-reflexes (and stretch reflexes) reduce in amplitude when vibration is applied, due to pre-synaptic inhibition of the Ia afferents (de Gail et al. 1966). In masseter, H-reflexes and stretch reflexes are potentiated when vibration is applied to the jaw-closing muscles (Godaux and Desmedt, 1975; Desmedt and Godaux, 1980). The proposed mechanism for this facilitation of the masseteric reflexes has been that there is a lack of pre-synaptic inhibition of the Ia afferents from masseter (Desmedt and Godaux, 1980). However, pre-synaptic inhibition of masseter Ia afferents has been demonstrated, and the findings of the current study suggest an alternative explanation for the facilitation of stretch and H-reflexes in the presence of vibration. A possible mechanism for the facilitation of H-reflexes in masseter in the presence of vibration is shown in Figure 23. In the motoneurons of limb muscles, Ia afferent input is preferentially distributed to small motoneurons, and produces larger EPSPs in smaller motoneurons due to their greater input resistance. Therefore it is the small motoneurons which participate in the H-reflex. With the application of vibration, the increased Ia input would tend to recruit more motoneurons, but the pre-synaptic inhibition of the Ia afferent input de-recruits the most recently recruited motoneurons (Desmedt and Godaux, 1980). Therefore the overall H-reflex, recorded using surface
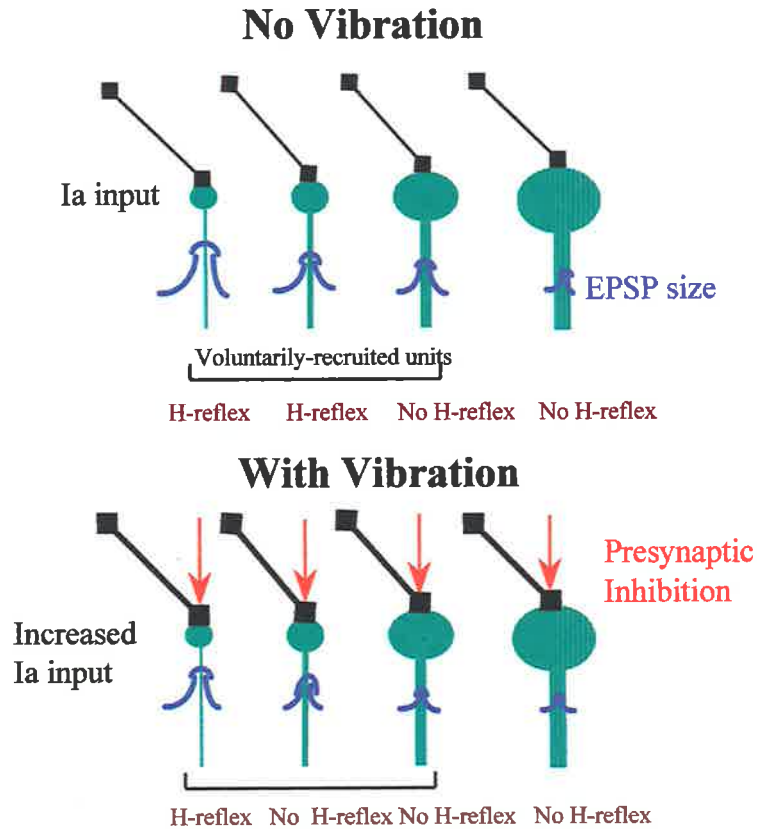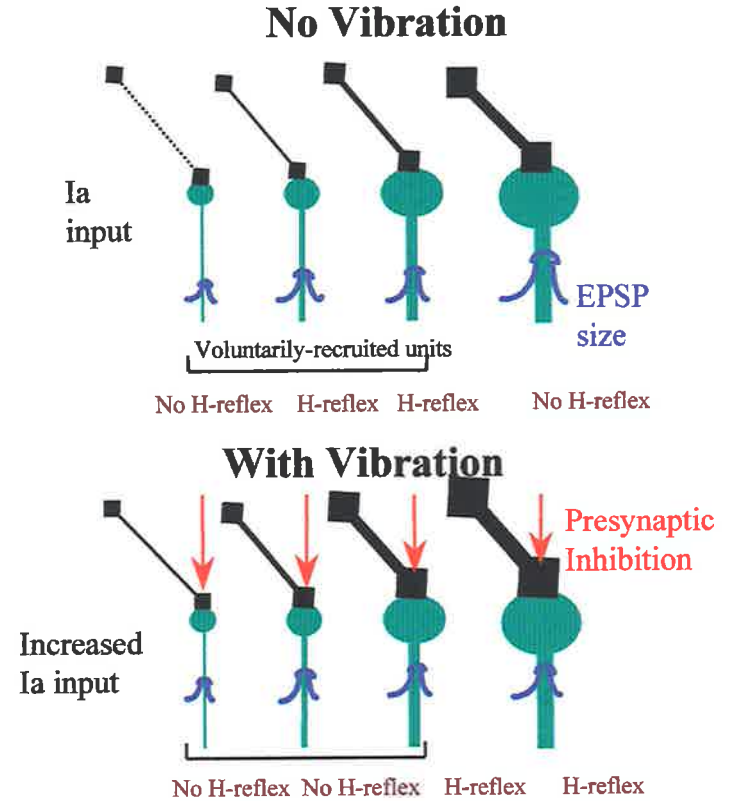
LIMB MUSCLES

No Vibration

Ia input

EPSP size

Voluntarily-recruited units

H-reflex   H-reflex   No H-reflex   No H-reflex

With Vibration

Increased
Ia input

Presynaptic
Inhibition

H-reflex   No   H-reflex   No H-reflex   No H-reflex

Figure 23. Model for Increase in Masseter H-reflexes with VIbration

In the limb muscles, Ia afferent input is equally distributed to all motoneurons. The resulting EPSPs are larger in the smaller motoneurons due to their greater input resistance. Therefore it is the smaller motoneurons which demonstrate an H-reflex. When vibration is applied, Ia input increases, but all motoneurons receive equal presynaptic inhibition, and net EPSP size decreases. Those motoneurons nearest the threshold (the large ones) lose the H-reflex, so total H-reflex amplitude decreases.

MASSETER

No Vibration

Ia
input

EPSP
size

Voluntarily-recruited units

No H-reflex   H-reflex   H-reflex   No H-reflex

With Vibration

Increased
Ia input

Presynaptic
Inhibition

No H-reflex   No H-reflex   H-reflex   H-reflex

In masseter, Ia input is more effective on large motoneurons. The resulting EPSPs are larger on the large motoneurons, despite their lower input resistance. When vibration is applied, all motoneurons receive equal presynaptic inhibition, and the H-reflex is lost in some smaller motoneurons. However, additional motoneurons are recruited into the H-reflex, resulting in an overall increase in the size of the H-reflex.

electrodes, decreases in amplitude. In masseter, greater effective Ia input onto voluntarily-activated large motoneurons results in their recruitment in the H-reflex. With the addition of vibration, Ia input increases and the small motoneurons now receive sufficient input to participate in the H-reflex. However, pre-synaptic inhibition of Ia afferent input results in the de-recruitment of the last-recruited motoneurons, which in this case is the small ones. The H-reflex overall increases due to the additional recruitment of large motoneurons. Note that this model assumes that pre-synaptic inhibition is equally distributed to all motoneurons, and that Ia afferent input is preferential to the large motoneurons.

8.4.2 Difficulty of eliciting H-reflexes in masseter

Two factors may contribute to the difficulty of eliciting H-reflexes in masseter. Firstly, Appenteng (1990) found that each masseter Ia afferent projected onto only about 10% of masseter motoneurons. However, considering that this study used stimulation of the whole masseteric nerve, the problem of limited distribution of the afferent input should have been overcome, if it is distributed to all motoneurons. Secondly, the largest diameter fibres in the masseteric nerve of the cat are the motor fibres (Morimoto and Nagashima, 1989b). If the same applies in humans, this could make H-reflexes difficult to elicit, as H-reflex responses would be blocked by antidromic impulses in the motor axons. Although this may explain the difficulty of eliciting H-reflexes, it cannot explain why H-reflexes were found in the large motor units rather than in the small motor units, nor why it was possible to elicit H-reflexes in the absence of M-waves. If thickness of

the motor axons is responsible for the difficulty eliciting H-reflexes, then this should first effect the large motor axons (supplying large motor units). If the motor axons are activated at lower stimulus intensities, then the recruitment curves for M-waves and H-reflexes would not fit the normal pattern found in the lower limbs, where H-reflexes are recruited at lower stimulus intensities than M-waves. Normal masseter recruitment curves have been found in this study (Chapter 5) and by previous authors (Godaux and Desmedt, 1975; de Laat and Macaluso, 1995).

The findings of this study suggest an alternative reason why masseter H-reflexes can be small in the surface EMG and be difficult to elicit, as the input from the muscle spindles is more effective on the large motoneurons. If the larger motoneurons receive a greater effective input from the muscle spindles, then these will be the motoneurons recruited into the H-reflex at the lowest stimulus intensities. However, the axons of large motoneurons are the thickest, and will be the first to be blocked by antidromic activity in the motor axons. It is likely that H-reflexes will only be recorded from these largest motoneurons when the stimulus is adjusted so that it passes more effectively through the Ia afferent fibres than through the motor axons. This may explain the occasions on which it was possible to record H-reflexes in stimulus-recruited units, which were very large. Thus the H-reflexes recorded from voluntarily-recruited motor units in this study are likely to have been from large motoneurons, but not usually from the largest motoneurons.

8.4.3 Stimulus-recruited units

Stimulus-recruited units were encountered in seven runs in the current study. Others have also reported stimulus-recruited units, in response to the stretch stimulus. Miles et al. (1995) and Calancie and Bawa (1984) reported stimulus-recruited units, although those units only participated in the long-latency component of the stretch reflex. Davies et al. (1993) found stimulus-recruited units in response to low-threshold electrical stimulation of the tibial nerve. Both the short latencies of the H-reflexes in the stimulus-recruited units and the large MacroRep amplitude indicate that these were large muscle units supplied by large motoneurons. In the current study, the recruitment of large units by the stimulus alone suggests that these large motoneurons must have received very strong Ia afferent input. The presence of stimulus- recruited units would tend to support the hypothesis that it is preferential distribution of Ia afferent input onto large motoneurons which is responsible for the development of H-reflexes in larger motor units. However, more work is needed in this area.

8.4.4 Functional Implications

A greater effective Ia input onto the large motoneurons has implications for the role of the muscle spindles in masseter. The role of the masseter muscle spindles in mastication is somewhat controversial. However, the findings of Morimoto and Nagashima (1989b) and Morimoto et al. (1989a) suggest that the muscle spindles do contribute to the control of the jaw-closing muscles during chewing. The distribution of the Ia afferents onto the large motoneurons may allow ready access to the large motoneurons, for

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strong, fast contractions of the masseter, as suggested by Dessem (1995). The findings of the current study would suggest that muscle spindle input would be used mostly in the development of high bite forces, such as during attack or defence situations, rather than in normal cyclic chewing. Dessem (1995), suggested that when the jaw muscles are used in aggressive biting behaviour, such as during defence or prey capture, the "fusimotor set" (Prochazka, 1989) will be high. This would make jaw muscle spindle afferents readily activated and would provide a short latency pathway to the masseter motoneurons. If the Ia input is more effective on the large motoneurons, as suggested by the findings of this study, then it will be the large, strong motor units that are preferentially recruited during such activities, giving the capacity to produce very large bite forces with the minimum effort.

In the limbs, the close association between muscle spindles and Type I muscle fibres, combined with the greater effective input from muscle spindle afferents onto small motoneurons, suggests a postural role for the muscle spindles. A more effective Ia input onto large motoneurons, as found in masseter, does not comply with this suggestion. Several studies have investigated the postural role of muscle spindles in masseter, with contrasting findings. The findings of the current study would support the contention by Yemm (1977b) and Miralles et al. (1987), that masseter has a greater role in force production rather than postural maintenance.

A preferential distribution of Ia afferents onto larger motoneurons may appear contradictory, given the close association between the muscle spindles and the Type I

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fibres in deep masseter (Burhanudin et al. 1996). This may almost suggest a degree of "inverse" functional compartmentalisation. However, the position of the muscle spindles in deep anterior muscles may be beneficial for sensing changes in muscle length of the masseter, as this area is furthest away from the temporomandibular joint.

The jaw is exposed to large vertical forces as the head accelerates up and down during the walking or running cycle (Lund, 1990), and a mechanism to control the posture of the jaw is needed to maintain the jaw in a stable position during such activities. The input from masseter muscle spindles onto large motoneurons may be necessary to maintain jaw posture during activities such as walking or running, if the size of the opening and closing forces imposed on the jaw are large. The high fatigability of the large motor units may be thought to preclude them from such activity, but the cyclic nature of walking may allow sufficient time for rest. Thus the finding that input from muscle spindles is more effective on large motoneurons may be consistent with such a dynamic postural role.

8.5   Summary

In this study the effectiveness of Ia distribution on simultaneously-active large and small motoneurons in human deep anterior masseter has been investigated. Large motoneurons were found to show H-reflexes more often than smaller motoneurons. Whether this is due to preferential distribution of Ia input or to pre-synaptic inhibition of Ia afferent input onto smaller motoneurons cannot be determined from this study,

remaining an area for future investigation. More effective Ia input onto large motoneurons may explain why H-reflexes are difficult to elicit in masseter, and why strong voluntary contractions are required to produce H-reflexes.

More effective distribution of Ia afferent input onto large motoneurons may suggest that the stretch reflex in masseter is more important in the development of large, fast forces, rather than in the maintenance of static posture.

9. Summary and Concluding Remarks

The findings of the studies described in the previous chapters can be summarised as follows:

Eliciting and Recording Masseteric H-reflexes.

H-reflexes can be elicited in masseter using a transmuscular stimulating technique, which is safe and comfortable for subjects. The H-reflexes and M-waves elicited using this technique respond to changes in stimulus intensity in a similar way to H-reflexes in other muscles. H-reflex onset latencies recorded using this technique are similar to those reported by other authors, using surface or needle stimulating techniques.

The H-reflex is more clearly demonstrated in the Macro EMG than in the surface EMG. H-reflexes can be recorded in individual motor units in masseter, although not all motor units demonstrate H-reflexes.

Recruitment of Masseter Motor Units During Slow Isometric Voluntary Biting.

Masseter motor units are recruited in order of increasing muscle unit size, as indicated by the amplitude of the MacroRep. There is a strong relationship between MacroRep amplitude and H-reflex latency, indicating that MacroRep can be used as an indicator of relative motoneuron size. Thus, recruitment of motoneurons in masseter occurs according to the size principle, during slow isometric contractions.

The variability in the contribution of masseter to the development of slow isometric biting ramps may be responsible for the variability in the force recruitment threshold of masseter motor units. The surface EMG recruitment threshold is a more reliable indicator of motor unit size than the force recruitment threshold.

Effectiveness of Ia Afferent Input on Masseter Motoneurons.

H-reflexes occur more frequently in the large motor units in masseter, indicating that the Ia afferent input is more effective on large motoneurons.

The finding that Ia input is more effective on large motoneurons in masseter may explain the small size and difficulty of eliciting H-reflexes in masseter, as the reflex volleys transmitted down the large motor axons will be the first to be blocked by antidromic activity, resulting from direct stimulation of the motor axons. Preferential distribution onto large motoneurons also explains the need for voluntary contractions in masseter when attempting to evoke H-reflexes, as the large motoneurons need to be voluntarily recruited before they can participate in the H-reflex. However, some large motor units (stimulus-recruited units) apparently receive sufficient Ia afferent input to be recruited by the stimulus alone.

The findings of the studies described do not allow conclusions to be drawn about the mechanisms responsible for the greater effective input of Ia afferents onto large motoneurons. Thus, either preferential input of Ia afferents onto large motoneurons, or

pre-synaptic inhibition of the input onto smaller motoneurons may explain the findings. The presence of large stimulus-recruited units would tend to suggest that preferential distribution onto large motoneurons is the more likely mechanism, but this remains an area for further investigation.

Limitations of the Study

In this study, all motor unit recording was undertaken in anterior masseter, and there is no certainty that the results can be generalised to other areas of the masseter. Masseter has been identified as a muscle containing neuromuscular compartments, which participate in different activities (Blanksma et al. 1992; van Eijden et al. 1993; Blanksma et al. 1995). If masseter behaves as a compartmentalised muscle, then the recruitment of motor units, and their responses in the H-reflex, may differ in different compartments. Similarly, the effectiveness of the Ia input on the motoneurons may differ in other areas of masseter, and the relationship between MacroRep amplitude and H-reflex latency has not been demonstrated.

All of the H-reflexes in these studies were recorded from the left masseter. However, it is likely that the results are applicable to the right side as well. Kossioni and Karkazis (1999) investigated the jaw jerk in left and right masseter and found that overall there were no significant differences in occurrence, latency, amplitude and duration. In individuals, variation between left and right were reduced when the subjects were voluntarily contracting masseter, as they were in the current study. Cruccu et al. (1992)

found that mean latency difference between left and right jaw jerk was only 0.13 ms. Thus the results from this study should be equally applicable to right or left masseter.

Electrode insertion in this study was into deep anterior masseter, which contains a high proportion of Type I muscle fibres (Eriksson, 1982) and a large muscle spindle population (Maier, 1979; Eriksson and Thornell, 1987; Rowlerson et al. 1988; Sciote, 1993) . It was not possible to determine the functional properties of the motor units, in order to determine whether the muscle fibres were Type I or Type II. The largest motor units are probably composed of large numbers of (small) Type II fibres. In this study, the larger motor units received more effective Ia afferent input. However, it is not possible to state whether this input occurred onto large Type I motor units as well.

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11.   Appendices

<u>**Name**</u>: Sheila Doreen SCUTTER

<u>**Present Position**</u>: Senior Lecturer, Faculty of Health and Biomedical Sciences, University of South Australia

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Qualifications

Bachelor of Applied Science
South Australian Institute of Technology
(Now University of South Australia)   1980

Honours(1) in Physiotherapy
South Australian Institute of Technology   1983

Master of Educational Studies
Flinders University of South Australia   1990

Journal Reviews
Australian Journal of Physiotherapy

Grants Assessment
ATN ARC Small Grants Assessor Network
Physiotherapy Research Foundation

Scholarships
I am currently holding an Australian Postgraduate Scholarship for my PhD studies.

Research Grants

Title	Granting Body	Amount of Grant
1998		
University of Adelaide Postgraduate Travel Prize	University of Adelaide	$400
1997		
The influence of for and aft isolators and swivel seating in trunk muscle performance, muscle activity and spinal creep in response to WBV in farmers. *Scutter SD, Fulton IJ, Grant ER*	Rural Industries Research and Development Corporation	$25,500
1996		
The effect of chronic exposure to vibration on jaw muscle reflexes. *Scutter SD and Türker KS*	University of South Australia	$14,000
The influence of tractor seating on trunk muscle performance, muscle activity and spinal creep in response to WBV in farmers. *Scutter SD and Fulton IJ*	University of South Australia Research Development Grant	$9000
RIRDC Travel Grant	RIRDC	$928
Physiotherapists in Rural and Remote Areas *Scutter SD and Sheppard LA*	Australian Physiotherapy Association Research and Development Fund	$1190
The effects of whole body vibration on trunk muscle performance	RIRDC	$22,500
1995		
The effects of various isoinertial training programs *Trott,P and Scutter,SD*	Physiotherapy Research Foundation	$5000
Introduction to Physiotherapy: An Evaluation *Carr JA and Scutter SD*	ANZAME	$400
The effects of whole body vibration in a simulated tractor driving position of flexion and rotation *Fulton IJ and Scutter SD*	University of South Australia Research Development Grant	$15,000
A model for undergraduate and postgraduate allied health education in rural and remote areas *Sheppard L and Scutter SD*	Rural Health Support, Education and Training Fund	$210,665

1994		
Introduction to Physiotherapy: an evaluation *Carr JA and Scutter SD*	ANZAME	$400
The repeatability of trunk muscle measurements using Isostation B200 *Scutter SD*	University of South Australia	$5000
The effects of vatious Isoinertial training programs *Trott P and Scutter SD*	University of South Australia	$5000

Refereed Publications

Scutter SD and Türker KS: (1999) Muscle Spindle Afferent Input to Motoneurons in Human Masseter. *J Neurophysiol* 82: 505-507

Scutter SD and Fulton IJ: (1999) The Effect of Seating Modifications on Back Pain in Farmers. RIRDC Final Report

Scutter SD and Türker KS: (1998) Recruitment stability in motor units of human masseter. *Muscle and Nerve* 21:1290-1298

Scutter SD and Türker KS: (1997) A new method for eliciting and studying H-reflexes in the human masseter. *Archives of Oral Biology.* 371-376

Türker KS, Yang J and Scutter SD: (1997) Tendon tap induces a single long-lasting excitatory relfex in the motoneurons of human soleus muscle. *Experimental Brain Research* 115, 169-173

Scutter SD, Türker KS and Hall R: (1997) Headaches and Neck Pain in Farmers.(1997) *Australian Journal of Rural Health.* 5, 2-5

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Scutter SD, Goold MA: (1995) Burnout in recently Qualified Physiotherapists in South Australia *Australian Journal of Physiotherapy.* 41: 115-118

Scutter SD: Why Do Students Enrol in the Bachelor of Applied Science in Physiotherapy? (1990) *Australian Journal of Physiotherapy,* 36: 23 - 28

Book Chapters

Türker KS, <u>Scutter SD</u>, Yang J, Jenkins M, Tucker KA and Abolfathi PP: Reflex control of masticatory muscles in man. In Excerpta Medica International Congress Series. Vol 1186 1999. Neurobiology of Mastication From Molecular to Systems Approach 337-353. Tokyo Nov 19-21 1998.

<u>Scutter SD</u> and Türker KS: A Method for Determining Muscle spindle afferent input to motoneurons. In Excerpta Medica International Congress Series. Vol 1186 1999. Neurobiology of Mastication From Molecular to Systems Approach. 266-269 Tokyo Nov 19-21 1998.

Scutter SD: Tractor driving and the low back- the effects of asn air cushion and a swivel seat on spinal movement during rotation. In Human Capital, Communications and Information Systems. Rural Industries Research and Development Corporation. 20-21, 1999

Conference Proceedings

<u>Scutter SD</u> and Türker KS: Muscle Spindle Input is More Effective on Large Motoneurons in Human Masseter. Neurobiology of Mastication. From Molecular to Systems Approach. Tokyo, November 1998.

<u>Scutter SD</u> and Türker KS: Ia Afferent Distribution to Motoneurons in Human Masseter. Society for Neuroscience 28 Annual Meeting Los Angeles, California, November 1998

Türker KS, Jenkins M, <u>Scutter SD</u> and Yang J: A method for quantifying reflex responses from the surface electromyogram. Australian Physiological and Pharmacological Society, Adelaide, October 1997

<u>Scutter SD</u> and Türker KS: A new method for eliciting and studying H-reflexes in the human masseter. Australian Neuroscience Society 16th Annual Meeting, Newcastle, 2 – 5 February 1997

<u>Scutter SD</u> and Türker KS: Recruitment order of masseter motoneurons during slow isometric voluntary contractions. Australian Physiological and Pharmacological Society, Adelaide, October 1997

Türker KS, Yang J and <u>Scutter SD</u>: How silent is the "silent period"? Australian Neuroscience Society. 16th Annual Meeting Adelaide January 28-31 1996

Scutter SD: The effects of WBV on farmers driving tractors. Farm Injury in Australia Conference, Wagga Wagga, 14-16 October 1994

Scutter SD: The effect of whole body vibration in farmers driving tractors. Prevention Strategies in Occupational Health and Safety Conference. University of South Australia, Adelaide. September 1994

Scutter SD: The Sacroiliac Joint in Low Back Pain and Pregnancy. Preconference Workshop, Australian Physiotherapy Association National Congress, Adelaide September 1992.

Scutter SD: The effects of isoinertial and isometric training programs on trunk muscle performance using Isostation B200, Australian Physiotherapy Association National Conference, Adelaide September 1992

Scutter SD: The effects of isoinertial and isometric training programs on trunk muscle performance using Isostation B200. Australian Physiotherapy Association National Conference, Adelaide 1992

Carr JA and Scutter SD: The evaluation of a new subject in the Bachelor of Applied Science in Physiotherapy, World Confederation of Physical Therapy, 11th International Conference, London 1991

Scutter SD: Muscle function recovery after arthroscopic medial meniscectomy Australian Physiotherapy Association National Conference Canberra 1986

11.2 Papers Resulting From this Thesis.

Copies of the following are included:

Scutter SD and Türker KS: (1997) A new method for eliciting and studying H-reflexes in the human masseter. *Archives of Oral Biology.* 371-376

Scutter SD and Türker KS: (1998) Recruitment stability in motor units of human masseter. *Muscle and Nerve* 21:1290-1298

Scutter SD and Türker KS: (1999) Muscle Spindle Afferent Input to Motoneurons in Human Masseter. *J Neurophysiol* 82: 505-507

Published

Scutter SD and Türker KS: A Method for Determining Muscle spindle afferent input to motoneurons. In Excerpta Medica International Congress Series. Vol 1186 pp 266 – 269, 1999. Neurobiology of Mastication From Molecular to Systems Approach. Tokyo Nov 19-21 1998.

In Press

Scutter SD and Türker KS (1999) Recruitment Order of Motoneurons Supplying Human Masseter. *Archives of Oral Biology.*

Pergamon

PII: S0003-9969(97)00029-0

A NEW METHOD FOR ELICITING AND STUDYING H-REFLEXES IN THE HUMAN MASSETER

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Summary—A non-invasive method is presented for transmuscular stimulation of the masseteric nerve, using a frame to apply a cathode to the mandibular notch and an anode to the inside of the mouth. The H-reflex response was recorded using surface, macro and single motor-unit (SMU) electromyography (EMG) from the masseter. The latency of the reflex response representing the H-reflex in SMUs was determined from the cumulative sum of the peristimulus time histogram. This latency was then corrected using a spike-trigger averaging technique, where the SMU spikes were used as triggers and the macro EMG recording as the source. SMU latencies for the H-reflex in masseter were in the range 5.9–8.8 msec, whereas H-reflex latencies for surface EMG varied between 5.4 and 6.4 msec. © 1997 Elsevier Science Ltd

INTRODUCTION

A variety of methods have been developed for stimulation of the masseteric nerve in order to elicit the H-reflex in masseter. Godaux and Desmedt (1975) applied direct electrical stimulation using bipolar needle electrodes and recorded the H-reflex with surface electrodes or subcutaneous needles. Macaluso and De Laat (1995a) stimulated the masseteric and deep temporal nerves using a monopolar needle electrode with a surface anode on the opposite cheek, and produced M-waves and H-reflexes in both masseter and temporalis. Although successful in producing H-reflex responses, these techniques are invasive and may be uncomfortable for the participants. Using these methods to stimulate the masseteric nerve it was necessary to insert one or two electrodes to a depth of 15–20 mm (Godaux and Desmedt, 1975; Macaluso and De Laat, 1995a), in an area which is in the vicinity of the pterygoid plexus. In addition, distortion of the needle electrodes during jaw movements is a distinct possibility. Once positioned, fine lateral movements of the electrodes in relation to the masseteric nerve are not possible, which may necessitate repeated insertions to achieve satisfactory results. We have now attempted to develop a stimulating technique that is non-invasive, flexible and effective for producing H-reflexes in masseter.

The method presented uses recordings of the H-reflex in single motor unit in masseter as well as the surface and Macro EMG. The H-reflex represented in single motor units has not, to the best of our knowledge, previously been studied in jaw-closing muscles, although this technique has been used extensively to investigate the reflex control of limb muscles. Previous investigations of the H-reflex in the masseter have relied on surface or subcutaneous multi-unit EMG (Fujii and Mitani, 1973; Mitani, 1974; Godaux and Desmedt, 1975; Fujii, 1977; Macaluso and De Laat, 1995a). However, the short latencies of the M-wave and H-reflex in masseter (about 2 msec and 6 msec, respectively) make these responses difficult to separate from each other and from the stimulus artefact. Recording the H-reflex in single motor units is beneficial as the action potentials are all-or-nothing events unaffected by stimulus artefact. Also, when using surface EMG or multi-unit needle electrodes, information is gathered from a whole range of motoneurones and it becomes difficult to ascertain the effect upon individual motoneurones.

MATERIALS AND METHODS

Fifteen healthy volunteers (4 males, 11 females, age range 20–40 years) participated in the experiments. All gave their informed consent. Ethical approval was obtained from the University of Adelaide Human Research Ethics Committee.

Participants were seated comfortably in a dental chair during the experiments, which usually lasted

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Abbreviations: EMG, electromyography(-gram).

for about 3 hr. The head and neck were maintained in a neutral posture and background noise was low.

Stimulating electrodes

The anode and cathode were incorporated into a U-shaped stainless-steel frame, shown in Fig. 1. The frame was held in position by a bite plate constructed of thermoplastic material, which was moulded to the shape of the individual's teeth and attached to a flat stainless-steel plate near the anode. The bite plate covered the entire occlusal surfaces of the upper and lower teeth. The cathode could be finely adjusted to locate the best stimulating position. The bite plate maintained the bite position, which is necessary because altering the bite position changes the firing pattern of the motoneurones (Eriksson *et al.*, 1984). When the jaw-closing muscles were relaxed the bite plate rested comfortably between the teeth.

Recording electrodes

One Teflon-insulated, bipolar, silver-wire single motor unit electrode and a single macro electrode were inserted to a depth of approx. 2 cm into the anterior portion of masseter using one 25 G needle. The insulation was stripped from the terminal 15 mm of the macro electrode. The wires for single motor units were completely insulated except for the tip. The needle was withdrawn leaving the three wires in the belly of the muscle. The EMG signal from single motor units was filtered at 200 Hz–5 kHz and the macro at 50 Hz–5 kHz. Bipolar surface electrodes were also placed over the anterior masseter on both sides of the face. The electrodes on the left gave a surface representation of the responses of masseter to the stimulation; those on the
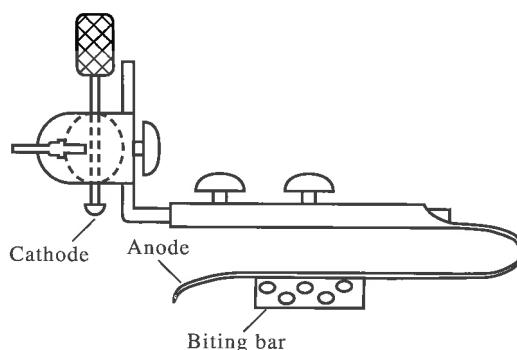

Fig. 1. Frame used for attachment of stimulating electrodes. The frame was inserted into the participant's mouth so that the anode was against the medial aspect of the left masseter, posterosuperior to the upper second molar. The position of the anode was maintained by the biting plate, which was held between the molars. Thermoplastic material was attached to the biting bar to allow accurate moulding to the participant's teeth. The cathode could be moved with six degrees of freedom and then fixed in place. The cathode was adjusted so that it pressed firmly into the mandibular notch.

right were used to give the participant feedback about the level of EMG activity.

Protocol

Maximum voluntary clenching activity was recorded by the rectified, filtered (D.C.-1 Hz) surface EMG of the right masseter while asking the individual to bite as hard as possible. The maximum of three attempts was accepted. The participant was then given visual feedback of the level of EMG activity required to produce about 10% of maximum activity. The anode and cathode were then positioned and the stimulus applied. While the biting level was held constant at about 10% of maximum, the effect of altering stimulus intensity on M-wave and H-reflex amplitude was recorded. A Digitimer Ltd Isolated Stimulator (Model DS2) with a constant-current device was used to apply the stimulus. Stimulus intensity was increased in increments of 1 V. This procedure was then repeated with the masseter relaxed.

The participants were then asked to control the discharge of one masseter motor unit at a steady frequency. With the help of audio-feedback, they were able voluntarily to regulate the firing rate of a particular motor unit so that it ran at a steady frequency, which was usually around 12–18 Hz. While the motor unit was firing steadily, the masseteric nerve was stimulated. Stimulus pulse width was 1 msec and was applied randomly at a frequency of 0.2–0.6 Hz. Stimulation was continued until the participant was no longer able to continue to fire the unit regularly. The surface EMG, macro EMG and single motor unit of the masseter were recorded on a video-tape for off-line analysis. Grounding was achieved by means of a lip-clip electrode (Türker *et al.*, 1988).

Analysis

The shape of the single motor unit action potentials was recognized using a template-matching algorithm (SPS-8701) that sent out a recognition pulse whenever it matched the shape of an action potential. The reflex response of one motor unit only was determined off-line. The chosen unit was always the one with the largest action potential. The motor-unit potentials were filtered to single out the shape and the amplitude of the unit of interest and depress the amplitudes of any others. This process reduces the chances of superimposition of other unit potentials on the potential of the unit of interest at the H-reflex latency. Such superimposition can interfere with the recognition of the unit potential at the M-wave or H-reflex latencies by the template-matching program, and hence reduce the estimated size of the reflex response.

The latency of the H-reflex measured in single motor units can be inaccurate, as the intramuscular delay will differ depending on the position of the recording electrode within the motor unit (Awiszus
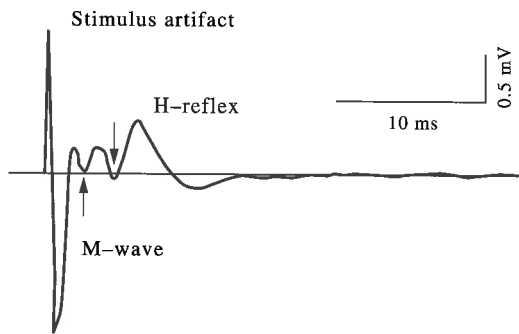
Fig. 2. Surface EMG of M-wave and H-reflex in masseter. The short latencies of the responses made accurate determination of latencies difficult in many examples. In this case, which was one of the clearest examples, the H-reflex occurred at a latency of 5 msec and the M-wave at 2.5 msec.

and Feistner, 1993). To determine accurately the latency of the H-reflex, the macro EMG was triggered by the spike of the single-fibre EMG. At least 400 triggers were averaged to produce the representation of the unit in the macro. The time between the onset of the macro EMG and the trigger spike was used to estimate the intramuscular delay. This was then subtracted from the latency measured by the single motor unit (Awiszus and Feistner, 1995). Surface representation of the unit was often small and not useful for latency correction.

The latency of the M-wave and H-reflex could be determined using both the surface EMG and the macro EMG. The macro recording was more useful as the stimulus artefact was smaller.

RESULTS

M-wave and H-reflex responses were recorded from 29 units in 15 experiments. Figure 2 shows an example of the surface EMG of the M-wave and H-reflex using the stimulation method described. In this experiment the M-wave and H-reflex were

clearly seen, but in many cases their proximity to each other and to the stimulus artefact made accurate determination of the reflex latency difficult. Where clear separation of the responses was achieved (six experiments), latencies were determined. Mean M-wave latency was 2.1 msec (range 1.7–2.4 msec) and mean H-reflex latency was 5.9 msec (range 5.4–6.4 msec).

Figure 3 shows an example of the peristimulus time histogram and cumulative sum (Ellaway, 1978) produced in a single motor unit. This figure shows that the latency of the H-reflex, as shown by the take-off point of the cumulative sum, was about 10.5 msec. Figure 4 shows the averaged macro EMG representation triggered by the firing of the single motor unit ($n = 400$), illustrating that the action potential of the single motor unit had arrived in the muscle 1.7 msec earlier than the action potential recorded by the single motor-unit electrode. Thus the correct latency for the H-reflex produced in this unit was 8.8 msec.

H-reflex latencies in single motor units, calculated from the onset of a rise in cumulative sum, ranged from 7.5–10.5 msec (mean 8.7 msec). When the latencies were corrected individually by spike-triggered average of the macro the resulting range was 5.9–8.8 msec (mean 7.0 msec).

In 12 experiments the size of the H-reflex recorded in the macro and surface EMG increased with increasing stimulus intensity. In six of these experiments the H-reflex amplitude decreased as stimulus intensity was further increased. In only two cases was it possible to completely eradicate the H-reflex by increasing stimulus intensity. In nine experiments the H-reflex could be produced at a lower stimulus intensity when the individual was biting at 10% of maximum than when the masseter was relaxed. Figure 5 shows the amplitude changes in the macro EMG of an experiment in which the H-reflex appeared at a lower stimulus intensity than the M-wave. The H-reflex amplitude increased in
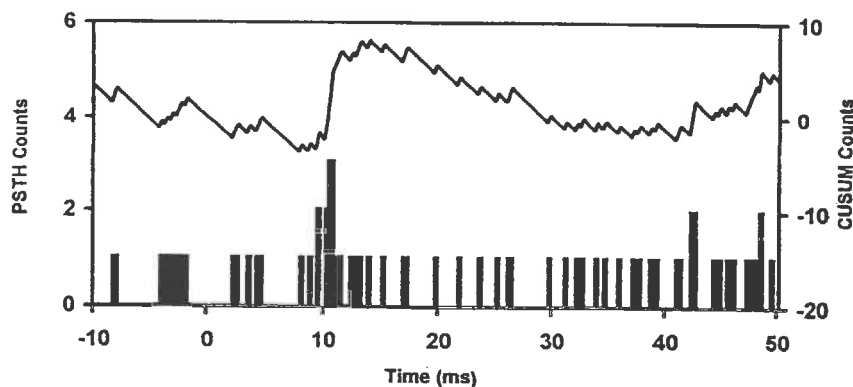

Fig. 3. Peristimulus time histogram (PSTH) and cumulative sum (CUSUM) showing H-reflex in a single motor unit in the masseter. The latency of the H-reflex, indicated by a rise in CUSUM, was 10.5 msec. Stimuli ($n = 114$) were delivered at time zero. Bin-width of the histogram was 250 μsec.

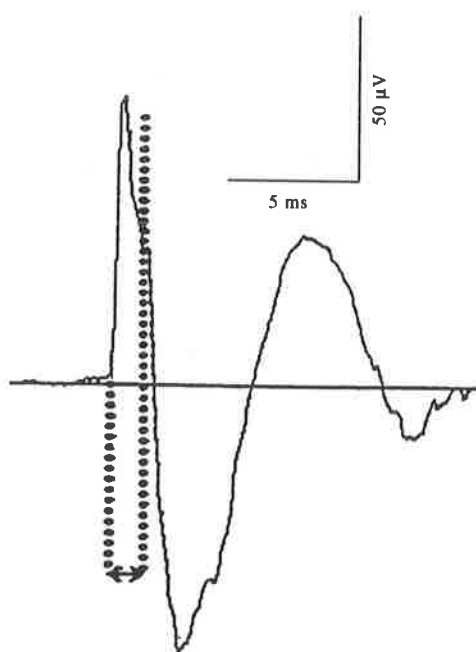intensity when they were biting at 10% of maximum than when relaxed (24 and 28 V).

Fig. 4. Macro representation of single motor unit (SMU) shown in Fig. 3. The averaged macro EMG was triggered by the unit spike ($n = 400$). The dotted line on the right is the time of the SMU trigger and the dotted line on the left is the time of arrival of the SMU potential in the muscle. The distance between these dotted lines illustrates that the SMU action potential arrived in the muscle 1.7 msec before the trigger spike was recorded by the SMU electrode. This 1.7 is the correction value which was subtracted from the H-reflex response latency determined from the cumulative sum.

size and then gradually decreased with increasing stimulus intensity. The M-wave gradually increased with increasing stimulus intensity. In this individual the H-reflex was produced at a lower stimulus
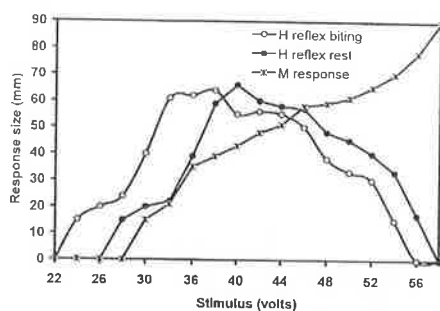

Fig. 5. Stimulus–response curves for the macro EMG. The H-reflex and M-wave recorded in the macro EMG are illustrated at different stimulus strengths. The threshold stimulus was lower when the participant was biting at 10% of maximum. As stimulus intensity increased, the size of the H-reflex gradually increased and then reduced, while the size of the M-wave increased.

DISCUSSION

Stimulating technique

We show that the H-reflex can be successfully elicited using a transmuscular stimulating technique, which is comfortable, reproducible and non-invasive. Macaluso and De Laat (1995b) compared surface, bipolar and monopolar techniques for stimulating the motor nerve to masseter. In the monopolar technique a needle electrode was inserted between the coronoid process and condyle of the mandible to a depth of 1.5 cm. The anode was a surface electrode placed on the opposite cheek. The bipolar technique involved inserting another needle 5 mm posterosuperiorly to the first (also used by Cruccu, 1989; Godaux and Desmedt, 1975). The surface technique involved a surface electrode over the mandibular notch and an anode on the opposite cheek. Surface electrodes were used to record from masseter and anterior temporalis. All three techniques were found to be effective in stimulating the motor nerves to masseter and temporalis. The monopolar needle was more stable than the bipolar and produced a greater amplitude of response more easily. Fujii and Mitani (1973) and Fujii (1977) used surface electrodes to stimulate the masseteric nerve. Electrode placement in these studies was superficial, with the cathode over the mandibular notch and the anode on the skin of the same side of the face, several centimetres away. Such placement is not ideal for stimulation of the masseteric nerve, which is situated at a depth of about 20 mm (Godaux and Desmedt, 1975). Furthermore the surface stimulation technique also activated cutaneous afferents and the facial nerve, leading to discomfort for the participant (Macaluso and De Laat, 1995b).

We used a transmuscular stimulating technique to produce a stable response with little or no discomfort to the participant. The placement of the anode inside the mouth allowed the stimulation to be localized to the masseteric nerve without much spread. When stimulating a nerve it is usually necessary to manipulate the position of the cathode in relation to the nerve, in order to obtain a maximal H-reflex with a minimal M-response. This fine adjustment of the cathode position is easily achieved using the stimulating technique we present. The cathode position is also reproducible throughout the extent of the experiment. With invasive stimulating techniques it is likely that the position of the cathode would be disrupted by mandibular movements.

Macaluso and De Laat (1995c) investigated the effect of the position of the surface recording electrodes on the M-wave recorded in masseter and

temporalis. They found that the optimal position for the surface-recording electrodes for masseter was over the anterior and inferior part of the muscle belly, and this position was used here for the insertion of the single motor-unit and macro electrodes. Our stimulating technique allowed for accurate, reproducible stimulation of the masseteric nerve, and for fine adjustment of the position of the cathode to evoke maximal H-reflex. Such fine lateral manipulation is obviously not possible with inserted stimulating electrodes, unless they are withdrawn and reinserted.

Recording responses

The very short latencies of the M-wave and H-reflex in masseter make clear separation difficult using surface recording techniques. The single motor-unit technique used in the present study allows clear separation of these two responses, and ensures that the stimulus artefact does not interfere with either of the responses. As the single motor-unit responses are all-or-nothing events, they are unambiguous and reliable. This technique will be useful in studying the distribution of Ia input to motoneurones, and for determining the motor units that contribute to the tonic vibration reflex (Desmedt and Godaux, 1980) in masseter.

The H-reflex and M-wave have previously been investigated in masseter: H-reflex latencies ranged from 5.4 msec (Godaux and Desmedt, 1975) to 5.91 msec (Macaluso and De Laat, 1995a) the latency of the H-reflex as measured here by surface EMG and macro EMG was similar. However, the single motor-unit latency measured by peristimulus time histogram was longer, due to the delay caused by conduction of the action potential through the muscle to the tip of the single motor-unit electrode. When the latency was corrected, the latency values were somewhat higher than those found by earlier investigators, although the lower part of the range was similar to values found using other techniques. Smaller motor units with longer latencies are likely to be under-represented in the surface recordings used by others, as the surface responses will be dominated by shorter-latency units presumably belonging to high recruitment-threshold motor units (Burke, 1981; Stuart and Enoka, 1983). The higher latencies occasionally found here emphasize the ability of this technique to record from motor units of different sizes and with different latencies. Our technique allowed us to follow the low-threshold units, those that are recruited first during normal activities.

There are a number of ways of determining that the response achieved was in fact an H-reflex (Hugon, 1973). An M-wave occurred infrequently in the unit followed (in three units), indicating that the response was not an F-wave. The reflex increased in size when the participant contracted the masseter and this would also not be expected to

occur with an F-response (Mayer and Feldman, 1967). The minimal stimulus intensity required to induce the H-reflex decreased with voluntary contraction. The H-reflex also increased in size with increasing stimulus intensity and then decreased as the stimulus was increased still further, and an M-wave developed (Fig. 5).

Conclusion

The method presented used transmuscular stimulation of the masseteric nerve, which provided for participant comfort and safety. Accurate positioning of the cathode and anode were achieved by use of a specially developed frame. The recording of the H-reflex response in single motor units and macro EMG will make possible a more detailed understanding of the reflex behaviour in the masseter muscle.

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