



# **REFLEX CONTROL OF HUMAN JAW MUSCLES BY PERIODONTAL MECHANORECEPTORS**

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## CONTENTS

<b>CONTENTS .....</b>	<b>1</b>
<b>ABSTRACT .....</b>	<b>6</b>
<b>DECLARATION.....</b>	<b>8</b>
<b>ACKNOWLEDGEMENT .....</b>	<b>9</b>
<b>LIST OF FIGURES.....</b>	<b>10</b>
<b>LIST OF TABLES.....</b>	<b>13</b>
<b>CHAPTER 1 INTRODUCTION AND STUDY AIMS.....</b>	<b>14</b>
<b>CHAPTER 2 LITERATURE REVIEW.....</b>	<b>19</b>
2.1 PERIODONTAL MECHANORECEPTORS.....	19
2.1.1 Physiological and Histological Properties .....	21
2.1.2 Central Projections .....	31
2.1.3 Functional Roles.....	35
2.2 JAW REFLEXES.....	37
2.2.1 Jaw Opening Reflex.....	38
2.2.2 Jaw Closing Reflex .....	44
2.2.3 Effects of Periodontal Mechanoreceptors on Jaw Reflexes .....	47

2.3 MASSETER.....	50
2.3.1 Structure .....	50
2.3.2 Motor Units and Muscle Fibres.....	53
2.3.3 Neuromuscular Compartments.....	61
2.4 FORCE DEVELOPMENT .....	63
2.4.1 Recruitment and the Size Principle .....	63
2.4.2 Firing Rate of Motor Units.....	70
2.4.3 Interaction between Recruitment and Rate Modulation.....	70
2.5 METHODOLOGICAL CONSIDERATIONS.....	72
2.5.1 Subjects.....	72
2.5.2 Stimulation Methods .....	73
2.5.3 Recording and Analysing Reflex Response .....	76
2.5.4 Recording the Size of Single Motor Unit.....	80

## **CHAPTER 3 STIMULUS PARAMETERS FOR ELICITING**

### **EXCITATORY MASSETERIC REFLEXES IN**

#### **HUMANS.....84**

3.1 INTRODUCTION.....	84
3.2 METHODS .....	85
3.2.1 Experimental Apparatus .....	85
3.2.2 Protocols.....	88
3.2.3 Surface EMG Analysis.....	89
3.2.4 Statistical Analyses .....	91

3.3 RESULTS .....	92
3.3.1 Preload vs No Preload .....	92
3.3.2 Reflex Responses of the Masseter to Different Stimulus Profiles.....	95
3.3.3 The Effect of a Number of Stimuli on the Incidence of Various Reflexes .....	97
3.4 DISCUSSION .....	99

## **CHAPTER 4 JAW REFLEX RESPONSES TO**

### **MECHANICAL STIMULATION OF A**

### **HUMAN TOOTH .....**

**104**

4.1 INTRODUCTION.....	104
4.2 METHODS .....	106
4.2.1 Experimental Apparatus .....	106
4.2.2 Experimental Protocol.....	113
4.2.3 Statistical Analyses .....	115
4.3 RESULTS .....	115
4.3.1 The Reflex Response Patterns Produced by Slowly-Rising Stimulation .....	115
4.3.2 The Reflex Response Evoked by Slowly-rising and Rapidly- rising Stimuli .....	121
4.4 DISCUSSION .....	124
4.4.1 Reflex Responses of SEMG of the Masseter and the Bite Force .....	124
4.4.2 Receptors and Pathways.....	126



#### 4.4.3 *The Reflex Pattern of the Bite Force vs the Background*

<i>Clenching Level.....</i>	128
-----------------------------	-----

## **CHAPTER 5 REFLEX RESPONSES OF SINGLE MOTOR**

### **UNITS IN HUMAN MASSETER TO**

### **SLOWLY-RISING STIMULATION OF A**

### **TOOTH .....131**

5.1 INTRODUCTION.....	131
-----------------------	-----

5.2 METHODS .....	133
-------------------	-----

5.2.1 <i>Periodontal Stimulation.....</i>	133
---	-----

5.2.2 <i>Single Motor Unit Recording .....</i>	133
--	-----

5.2.3 <i>Experimental Protocol.....</i>	136
---	-----

5.2.4 <i>Data analyses .....</i>	138
----------------------------------	-----

5.3 RESULTS .....	141
-------------------	-----

5.3.1 <i>Reflex Responses of Single Motor Units.....</i>	141
--	-----

5.3.2 <i>Reflex Responses of Different-Sized Single Motor Units.....</i>	144
--	-----

5.3.3 <i>Firing Frequency of Different-Sized Single Motor Units.....</i>	149
--	-----

5.3.4 <i>Force Recruitment Threshold of Single Motor Units .....</i>	149
--	-----

5.4 DISCUSSION .....	153
----------------------	-----

5.4.1 <i>Distribution of Synaptic Inputs from Periodontal</i>	
---	--

<i>Mechanoreceptors to the Motoneuron Pool of Human Masseter....</i>	153
--	-----

5.4.2 <i>The Effect of Discharge Frequency.....</i>	155
---	-----

5.4.3 <i>Methods for Determining the Size of Single Motor Units .....</i>	157
---	-----

<b>CHAPTER 6 CONCLUDING REMARKS .....</b>	<b>160</b>
<b>CHAPTER 7 REFERENCES.....</b>	<b>169</b>
<b>CHATER 8 APPENDIX .....</b>	<b>220</b>
8.1 CURRICULUM VITAE.....	220
8.2 PUBLISHED PAPERS RESULTING FROM THIS THESIS .....	224

## ***ABSTRACT***

It is well known that a brisk tap stimulus applied to the tooth induces a reflex inhibition of the jaw closing muscles. In contrast, the presence of reflex excitation is more controversial. In one study, where the reflex excitation of the jaw closing muscles could be recorded using a push stimulus on a tooth, the likelihood for eliciting an excitatory reflex response was about 40% (Brodin *et al.*, 1993b). In order to determine what factors affect the outcome of the reflex response of the jaw closing muscles to periodontal mechanoreceptive stimulus, in this study, the reflex responses of the human masseter were investigated by applying force using different stimulus profiles. It was shown that when the force profile had little or no fast component, the likelihood of eliciting an excitatory periodontal masseteric reflex increased. It is concluded that the shape of the stimulus profile, the location of the stimulating probe and the presence of preload are the main factors that determine the excitatory reflex response of the jaw closing muscles.

In contrast to the large number of studies using surface electromyography (SEMG) of the masseter (Van Der Glas & van Steenberghe, 1981,1988; Van Der Glas *et al.*, 1985; Bonte *et al.*, 1986; Bonte & van Steenberghe, 1989,1991; van Steenberghe *et al.*, 1989,1991; Brodin *et al.*, 1993b; Louca *et al.*, 1996a), the reflex changes in the bite force induced by a tooth stimulus have not been studied systematically. To determine whether the masseter muscle represents the effective changes in bite force in response to a tooth stimulus, the SEMG of the masseter and the bite force were recorded

simultaneously. The results showed that the pattern of reflex response in bite force was different from that in the SEMG of the masseter. Therefore, the reflex change in the masseter muscle does not give a good representation of reflex changes of bite force in humans. This result suggests that the net response of all jaw muscles is best expressed by the averaged bite force.

The distribution of periodontal mechanoreceptor input to various-sized motoneurons in the human masseter has not previously been studied. In this study, the reflex responses of different-sized single motor units (SMUs) to tooth stimulation were investigated. In the human masseter, the SMUs of larger size were found to have more excitatory and less inhibitory reflex responses than those of smaller size. The finding demonstrates that the inputs from the periodontal mechanoreceptors are not distributed equally to the masseteric motoneurons.

The results of these studies support the theory that the periodontal mechanoreceptors can induce both excitatory and inhibitory reflexes on the jaw closing muscles. The excitatory reflex becomes dominant when a smooth force is applied with preload. Care should be taken when using SEMG of the masseter to indicate the reflex response of all jaw muscles. The finding of periodontal mechanoreceptor input favouring the larger SMUs in the masseter suggests that, during normal chewing, the excitatory periodontal mechanoreceptor input may help the jaw closing muscles develop fast and powerful forces to overcome food resistance.

### ***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.

Signed:

Date:

02/02/2000

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## LIST OF FIGURES

<b>Figure 2.1</b>	A tooth and its periodontal tissues .....	20
<b>Figure 2.2</b>	Morphological illustration of periodontal mechanoreceptors .....	25
<b>Figure 2.3</b>	The central projection of periodontal mechanoreceptors .....	34
<b>Figure 2.4</b>	Diagrammatic illustration of the human masseter.....	51
<b>Figure 2.5</b>	Motor unit of human jaw muscle and limb muscle .....	57
<b>Figure 3.1</b>	Diagrammatic illustration of experimental apparatus .....	87
<b>Figure 3.2</b>	SEMG and its CUSUM .....	90
<b>Figure 3.3</b>	The effect of preload on the reflex responses of ipsilateral masseter .....	93
<b>Figure 3.4</b>	Different stimulus profiles and the reflex response of ipsilateral masseter .....	96
<b>Figure 3.5</b>	The effect of the number of stimuli on the percentage success of various reflexes .....	98
<b>Figure 4.1</b>	Diagrammatic illustration of experimental apparatus .....	108
<b>Figure 4.2</b>	Reflex responses of bilateral masseter muscles to slowly-rising stimulation .....	110

<b>Figure 4.3</b> Reflex response of bite force in response to a slowly-rising stimulus .....	112
<b>Figure 4.4</b> Reflex patterns of SEMG and bite force to a slowly-rising stimulus .....	117
<b>Figure 4.5</b> The ratio of the reflex bite force increase and decrease vs the background bite force level.....	120
<b>Figure 4.6</b> Different reflex responses produced by slowly-rising and rapidly-rising stimulus.....	122
<b>Figure 5.1</b> Diagrammatic illustration of the recording of single motor unit activity.....	135
<b>Figure 5.2</b> Measurement of force recruitment threshold of single motor units .....	137
<b>Figure 5.3</b> PSTH of a single motor unit.....	139
<b>Figure 5.4</b> Reflex responses of a single motor unit to slowly-rising tooth stimulation.....	143
<b>Figure 5.5</b> Reflex responses of a pair of different-sized single motor units to slowly-rising tooth stimulation .....	146
<b>Figure 5.6</b> Relationship between reflex response and MacroRep amplitude .....	148



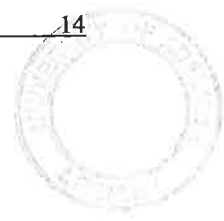
**Figure 5.7** Force recruitment threshold and MacroRep amplitude..... 150

**Figure 5.8** Reflex responses and force recruitment thresholds of single  
motor units ..... 152

**Figure 6.1** A speculative wiring diagram of periodontal  
mechanoreceptors and jaw muscles ..... 164

***LIST OF TABLES***

<b>Table 3.1</b>	Reflex responses as force applied with No Preload and with Preload.....	94
<b>Table 3.2</b>	Reflex responses to different stimulus profiles.....	95
<b>Table 4.1</b>	Reflex responses of SEMG and bite force to a slowly-rising stimulus .....	116
<b>Table 4.2</b>	Individual reflex responses of SEMG and bite force.....	118
<b>Table 4.3</b>	SEMG and bite force in response to a rapidly-rising stimulus .....	121
<b>Table 4.4</b>	Reflex responses of the bite force to slowly- and rapidly-rising stimuli.....	123
<b>Table 5.1</b>	Reflex responses of SEMG and bite force to slow-rising tooth stimulus .....	142
<b>Table 5.2</b>	Reflex response of single motor units.....	142
<b>Table 5.3</b>	Reflex responses of different-sized single motor units.....	145



## **CHAPTER 1**

### **INTRODUCTION AND STUDY AIMS**

The masticatory muscles move the jaw and create reaction forces in the teeth and temporomandibular joints. They also stimulate the skeletal tissue and influence craniofacial growth. From a clinical point of view, the consequences of their action are pertinent to any surgical or orthodontic corrections made to the craniofacial skeleton, prosthetic changes to dental occlusion, and the treatment of musculoskeletal disorders (Brand & Isselhard, 1994).

The basic rhythm of mastication is set by the central pattern generator (Dellow & Lund, 1971). In the absence of sensory inputs, mastication is highly inefficient (Kubo *et al.*, 1981) and even dangerous to the masticating organism. Sensory feedback from a variety of cutaneous, intraoral, periodontal, joint and muscle receptors interacts with the central nervous system at several levels to adapt the chewing pattern to the characteristics of the food. This is a source of the variability in the pattern of mastication (Lund, 1991; Ottenhoff *et al.*, 1992a,b). In addition, the generation of interocclusal forces during chewing demands sophisticated neural control, reliant to a great extent upon sensory feedback (Lund & Lamarre, 1973; Kennet & Linden, 1987). This interdependence indicates the importance of both the central pattern generator and feedback from receptors in forming the neurological basis of mastication.

The periodontal mechanoreceptor system is one of these sensory feedback systems and provides a major source of feedback when a tooth is mechanically displaced (Hannam, 1982). Most periodontal mechanoreceptors are sensitive to the amplitude or rate of a force applied to the crown of the tooth (Ness, 1954; Hannam, 1969; Linden & Millar, 1988a). Two populations of periodontal mechanoreceptors, slowly adapting and rapidly adapting, have been identified by their different physiological characteristics (Pfaffmann, 1939; Ness, 1954).

It is a well-known phenomenon that a strong and brisk mechanical stimulation of the crown of a tooth elicits a jaw opening reflex response in animals (Hannam & Matthews, 1969). Reflex inhibition of jaw closing muscles (masseter, temporalis and medial pterygoid) and reflex excitation of jaw opening muscles (mainly digastric), are attributed to the mechanism underlying the jaw opening reflex (for review: Matthews, 1975). The jaw opening reflex, therefore, has been generally considered to be protective and analogous to the withdrawal reflex, preventing damage to the soft tissues of the mouth. Brisk taps were used to elicit reflex responses in that study. Since this kind of stimulus can spread to distant mechanoreceptors, the role of the periodontal mechanoreceptors cannot be conclusively evaluated from such studies. More definite conclusions on the role of the periodontal mechanoreceptors can be made from a human study where ramp forces are applied to natural teeth (Bonte, 1993; Brodin *et al.*, 1993b).

In contrast to the evidence for inhibitory influences of the periodontal mechanoreceptors on jaw closing muscles (for review: Matthews, 1975), the presence

of excitatory effects of periodontal mechanoreceptors on jaw closing muscles is controversial (Bonte, 1993). Even though studies have indicated that using slowly-applied forces can initiate excitatory reflex responses from the human masseter muscles (Brodin *et al.*, 1993b; Türker *et al.*, 1994), other researchers have produced different reflex responses using similar force profiles (Bonte, 1993; Louca *et al.*, 1996a). The conclusion in the latter studies was that periodontal mechanoreceptors have only inhibitory effects on jaw closing muscles, performing a protective role (Louca *et al.*, 1996a). Therefore, one aim of the present study is to investigate the factors that can influence the reflex effects of periodontal mechanoreceptors on a human jaw closing muscle (masseter), using surface electromyography (SEMG) as an indicator of the reflex response.

Although SEMG has been widely used for reflex studies, interpreting the SEMG can be difficult (Widmer & Lund, 1989). An increase following a decrease in SEMG records can simply be a cluster of delayed action potentials resulting from the preceding inhibition (Miles *et al.*, 1987; Bonte & van Steenberghe, 1989). This change in SEMG may be wrongly described as an excitatory connection of the stimulated afferent to the motoneurons (Bonte & van Steenberghe, 1989; Awiszus *et al.*, 1991). SEMG also has one other major shortcoming, in that a large postsynaptic potential (PSP) shadows a later PSP since many of the active motoneurons discharging in response to the earlier PSP can no longer fire for a further interspike interval (Türker *et al.*, 1997).

The most reliable measure of the output of the masticatory system to a stimulus is the change in the force exerted between the teeth. Whether periodontal mechanoreceptors serve as positive feedback to the jaw muscle activity can be clearly demonstrated by the reflex change of bite force. Therefore, the second aim of the current study is to investigate the reflex effects of periodontal mechanoreceptors on bite force.

In order to understand the contribution of periodontal mechanoreceptors to the control of the masticatory muscles, the activity of single motor units (SMUs) has been investigated systematically in this study. It is well known that the orderly recruitment of motor units according to the size principle is a general rule of motor unit activity (Henneman *et al.*, 1965b). Deviations from this recruitment order can occur under conditions in which the motoneuron pool is the target of certain peripheral inputs (Burke *et al.*, 1970; Burke, 1991; Semmler & Türker, 1994; Scutter & Türker, 1999). In particular, several studies demonstrate that certain cutaneous inputs to the motoneuron pool disrupt the normal order of recruitment (Kanda *et al.*, 1977; Garnett & Stephens, 1980, 1981; Kanda & Desmedt, 1983). Since functionally and morphologically, periodontal mechanoreceptors are similar to cutaneous receptors, they may have similar effects on the motor control of jaw muscles. However, there is no information regarding the distribution of periodontal mechanoreceptor input to the motoneuron pool of the jaw muscles. The final aim of this study is to investigate whether the input of periodontal mechanoreceptors is distributed differentially to the different-sized motoneurons in the human masseter.

This study is focused on the influence of periodontal mechanoreceptor on the reflex control of the human jaw muscles. The findings of the study may be helpful in the understanding of the functional connections between the jaw muscles and periodontal mechanoreceptor system, which is necessary to elucidate the process of mastication and its underlying mechanisms. Unless the details of the neural mechanisms that control the motor functions of the masticatory system in health and disease are thoroughly understood, the diagnosis and treatment of masticatory dysfunctions and denture technology will lack a neurophysiological basis.

## **CHAPTER 2**

### **LITERATURE REVIEW**

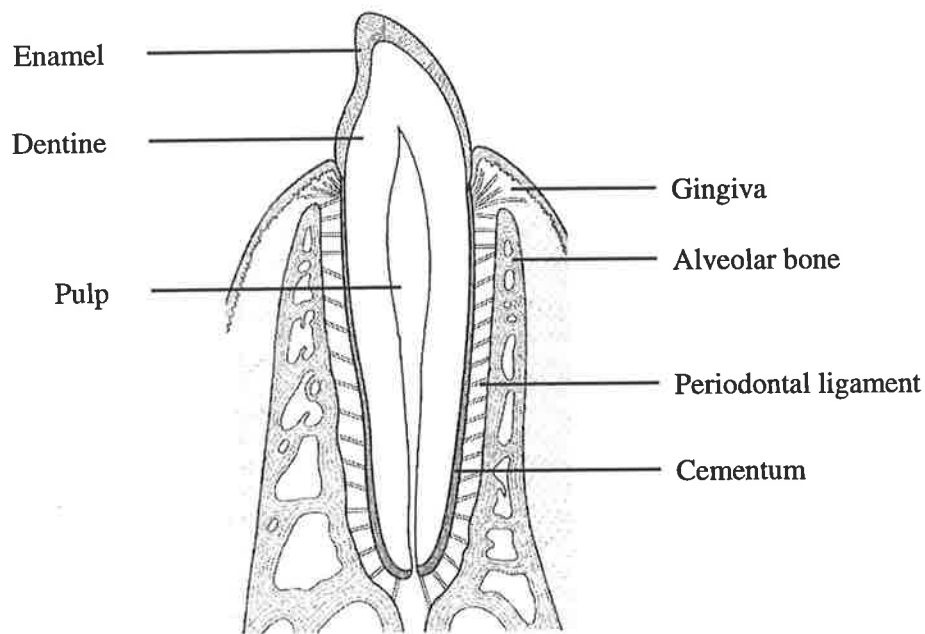
#### **2.1 PERIODONTAL MECHANORECEPTORS**

Periodontium consists of different tissues investing and supporting the teeth and includes the gingiva, cementum, periodontal ligament and alveolar bone (Rateitschak *et al.*, 1989)(Figure 2.1). Within the periodontal tissues, there are receptors that respond to forces applied to the teeth, and have been named 'periodontal mechanoreceptors' (Ness, 1954; Hannam, 1982; Linden, 1990). During mastication, periodontal mechanoreceptors are stimulated by the forces developed between the teeth and send messages to the central nervous system to modify the activities of the jaw muscles (Lund, 1991; Abbink *et al.*, 1998).

The activity of receptors that respond to forces applied to the teeth and their supporting structures has been reported by many workers studying a variety of species, and recordings have been made from a number of sites:

- peripheral nerves (Pfaffmann, 1939; Ness, 1954; Hannam, 1969; Sakada & Kamio, 1970,1971; Johansson & Olsson, 1976; Hannam & Farnsworth, 1977; Cash & Linden, 1982b; Karita & Tabata, 1985; Millar *et al.*, 1989; Loescher & Robinson, 1989b);
- trigeminal ganglion (Beaudreau & Jerge, 1968; Mei *et al.*, 1977; Appenteng *et al.*, 1982; Cash & Linden, 1982a; Linden & Scott, 1989a; Linden *et al.*, 1994);





**Figure 2.1 A tooth and its periodontal tissues**

Adapted from *The Periodontal Ligament in Health and Disease 2<sup>nd</sup>* (1995).

Edited by Berkovitz BKB, Moxham B, and Newman HN.

- mesencephalic nucleus of the fifth nerve (Jerge, 1963a; Linden, 1978; Amano & Iwasaki, 1982; Cash & Linden, 1982a; Passatore & Filippi, 1983; Linden & Scott, 1989a,b; Linden *et al.*, 1994; Passatore *et al.*, 1996);
- trigeminal nuclei (Jerge, 1963b; Olsson *et al.*, 1986,1988; Tabata & Karita, 1991a,c);
- thalamus (Karita & Tabata, 1991; Tabata & Karita, 1991b);
- cerebral cortex (Lund & Sessle, 1974; Mei *et al.*, 1977; Tabata & Karita, 1986).

Most of these studies were on anaesthetised animals. However, there are some studies in which microelectrodes have been used to record from the mandibular nerve (axon of periodontal mechanoreceptor) in humans by mechanically stimulating the crown of a tooth (Johansson & Olsson, 1976; Trulsson *et al.*, 1992; Trulsson, 1993a; Trulsson & Johansson, 1994).

### ***2.1.1 Physiological and Histological Properties***

The periodontal mechanoreceptors can be stimulated when the tooth is moved within the alveolar bone. The electrophysiological response properties of the receptors that respond to a force applied to a tooth were first studied by Pfaffmann (1939). In this section the physiological and histological properties of the innervation of the periodontal mechanoreceptors will be described.

### ***Adaptation time***

Pfaffmann (1939) was the first researcher to record periodontal mechanoreceptor activity from the dental branches of the maxillary division of the fifth nerve in the cat. According to the adaptation time associated with a prolonged stimulus, periodontal mechanoreceptors have generally been classified into two basic types - *rapidly adapting* receptors which fire only a short burst of impulses when they are stimulated, and *slowly adapting* receptors which fire through the period of stimulation (for reviews: Anderson *et al.*, 1970; Hannam, 1982; Linden, 1990; Linden *et al.*, 1995). It has been assumed that adaptation of the firing frequency can be of value in the sensory discrimination of forces applied to the teeth (Bonte, 1993).

Hannam (1969) considered that adaptation characteristics can be attributed to the spatial location of the receptor within the periodontal tissues, rather than to the presence of morphologically distinct groups of nerve endings. More evidence for this hypothesis was found in the observation that many of the receptors have intermediate response characteristics and thus do not fall easily into the two types of response characteristics (for review: Linden, 1990). A method has been developed to puncture a thin layer of bone overlying the labial aspect of the mandibular canine tooth and it enabled mechanical and electrical stimulation of the periodontal mechanoreceptors in the cat (Cash & Linden, 1982b; Linden & Millar, 1988b; Millar *et al.*, 1989; Millar & Linden, 1994). It has been found that all identified periodontal receptors respond to tension but not to compression of that part of the ligament in which they lay. In addition, a graded response in adaptation rate from fulcrum to apex was observed with

the more slowly adapting receptors situated in the apical part of the ligament, and the more rapidly adapting receptors situated in an area close to the fulcrum.

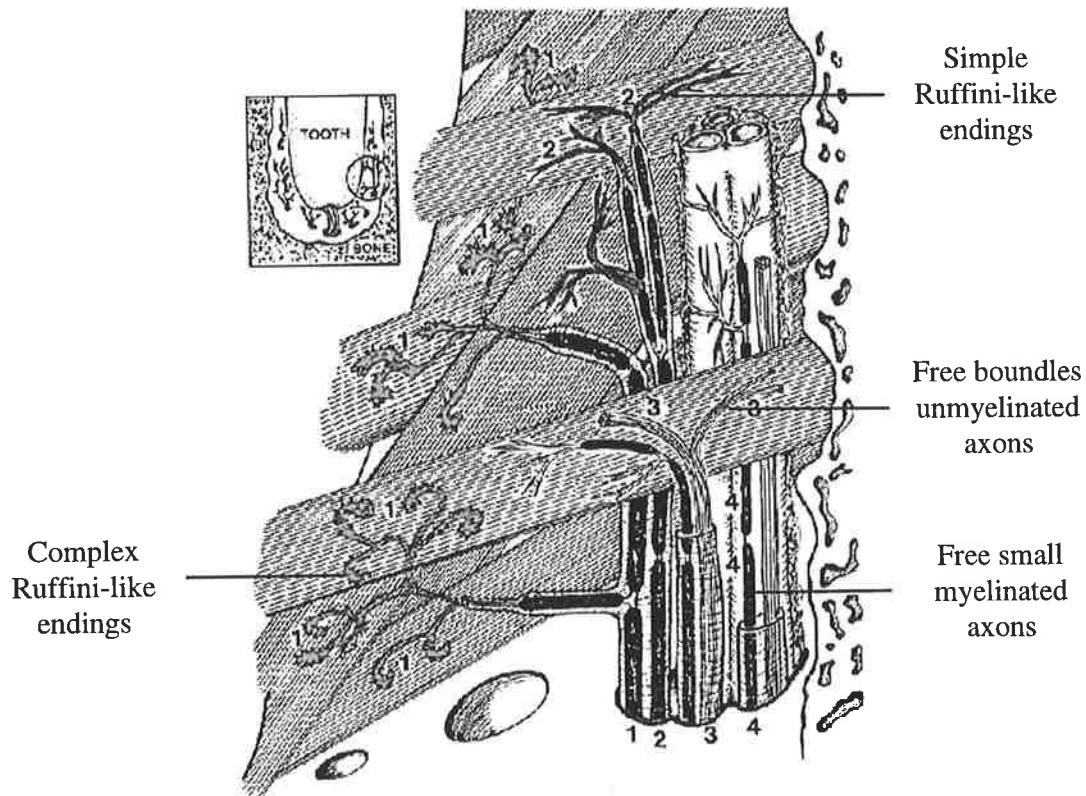
### ***Morphology of periodontal mechanoreceptors***

The nerves supplying the periodontal tissues have been studied histologically in humans (Maeda *et al.*, 1990; Lambrichts *et al.*, 1992,1993), monkeys (Byers & Dong, 1989), cats (Byers & Dong, 1989; Millar *et al.*, 1989; Linden *et al.*, 1994; Millar & Linden, 1994), rats (Byers, 1985; Byers & Dong, 1989) and other animals (Lewinsky & Stewart, 1936; Everts *et al.*, 1977; Freezer & Sims, 1989).

The trigeminal nerve innervates the periodontal mechanoreceptor from either its maxillary nerve or inferior alveolar branches. The nerve fibres run from the apical region of the root towards the gingival margins and are joined by small bundles of fibres that enter the ligament laterally through foramina in the alveolar bone. The fibres that enter from the alveolar plate divide into two bundles of approximately equal size, one turning peripherally towards the gingival margin and the other towards the apex of the root (Lewinsky & Stewart, 1936; Rapp *et al.*, 1957). Both large and small diameter nerve fibres have been described in the periodontal ligament, the large fibres being myelinated and the smaller being either myelinated or nonmyelinated (Byers, 1985). This observation prompted the suggestion that the large diameter fibres are concerned with touch sensation, whereas the smaller fibres are concerned with pain (Anderson *et al.*, 1970; Byers, 1985; Byers *et al.*, 1986; Linden, 1990). These fibres ultimately lose their myelin sheaths and terminate as neural endings (Byers, 1985).

Many different types of nerve endings have been described in the periodontal ligament eg. spindle-like, knob-like, Meissner-like, lamellated Vater-Pacini-like, Ruffini-like and free nerve endings (for reviews: Hannam, 1982; Linden, 1990; Linden *et al.*, 1995). Studies in animals have demonstrated a consistent occurrence of Ruffini-like endings in close relation to the collagen fibres (Byers, 1985; Byers *et al.*, 1986; Byers & Dong, 1989). By injecting tritiated amino acids into the trigeminal ganglion of rats, periodontal receptors were labelled by Byers (1985). In that study, four types of neuritis labelled from the trigeminal ganglion were described: (1) large complex Ruffini-like endings with finger-like extensions; (2) small simple Ruffini-like endings lacking neural fingers; (3) free bundles of unmyelinated axons; and (4) free, small myelinated axons (Byers, 1985)(see Figure 2.2).

It also has been assumed that the slowly adapting and rapidly adapting receptors form two morphologically distinct groups. They have been likened to receptors with similar adaptation properties found in the skin - ie. slowly adapting type II Ruffini endings and rapidly adapting Meissner corpuscles and other lamellated endings (Hannam, 1982).



**Figure 2.2 Morphological illustration of periodontal mechanoreceptors**

This diagram shows receptors among periodontal ligament and was adapted from Byers MR (1985). (1) complex Ruffini-like endings; (2) simple Ruffini-like endings; (3) free bundles of unmyelinated axons; (4) free, small myelinated axons.

From the combined studies in which periodontal ligament mechanoreceptors were studied physiologically, located, marked, and then subjected to histological investigation (Millar *et al.*, 1989; Linden *et al.*, 1994), it has been shown that Ruffini-like nerve endings respond to forces applied to a tooth and thus are considered as functional mechanoreceptors (Millar *et al.*, 1989). Such terminal endings have also been described in the periodontal ligament of humans (Maeda *et al.*, 1990; Lambrechts *et al.*, 1992). Griffin and Malor (1974) suggested that there are 2 types of encapsulated mechanoreceptors in the human periodontal ligament. One of these is a discrete unit consisting of an encapsulated myelinated fibre which loses its myelin sheath and divides into many small unmyelinated terminal, neural endings. The other type is more complex, consisting of three or more nerve fibres which lose their myelin sheaths and encircle one another with their terminal branches (Griffin & Malor, 1974). Assuming that both rapidly-and slowly adapting mechanoreceptors may be found in the periodontal ligament, it has been proposed that the discrete encapsulated endings may be associated with rapidly adapting neural responses, and that the more complex endings, responding more tonically to changes in periodontal tension, may be responsible for slowly adapting events (Griffin, 1972; Griffin & Malor, 1974).

Nerve terminals in the human periodontal ligament were also investigated by immunohistochemistry for neurofilament protein (NFP) and glia-specific S-100 protein (Maeda *et al.*, 1990). The human periodontal ligament was found to be innervated by NFP-immunoreactive nerve fibres, and contained free and specialised nerve endings. The specialised nerve endings consisting of thick nervous elements were classified into four types as follows: 1) Ruffini-like endings were found mainly

around the root apex; 2) coiled nerve endings were located in the mid-region of the periodontal ligament; 3) spindle-shaped types, and 4) expanded nerve endings, both rarely found near the root apex. The region-specific distribution of various nerve terminals demonstrated in this study seemed to be suited for receiving mechanical stimuli applied to the teeth from various directions (Maeda *et al.*, 1990). The ultrastructure of sensory nerve endings in the human periodontal ligament from extracted teeth was studied using serial sections and three types of nerve endings were found: free nerve endings, Ruffini-like endings, and lamellated corpuscles (Lambrichts *et al.*, 1992). The free nerve endings are regarded as receptors for pain and heat; the Ruffini receptors are thought to be mechanoreceptors of the slowly adapting type; the lamellated endings might be mechanoreceptors of the rapidly adapting type (Lambrichts *et al.*, 1992).

The above studies provide an overall understanding of the anatomy of the innervation of the periodontal supporting tissues. Due to differences in the species investigated (Sato *et al.*, 1992) and the limitations of the methodologies used (Linden *et al.*, 1995), the relationship between morphology and function of periodontal mechanoreceptors is still speculative.

### ***Force threshold level***

The threshold force which is needed to elicit a response from periodontal mechanoreceptors has been studied in animals (for reviews: Anderson *et al.*, 1970; Hannam, 1982; Linden, 1990; Linden *et al.*, 1995). Forces between 1-2g (0.01-0.02 N) are sufficient to evoke a response from most of the slowly adapting receptors, whereas,



rapidly adapting receptors have higher thresholds (Pfaffmann, 1939; Hannam, 1969). The threshold of more rapidly adapting receptors has been reported to be in terms of tens of grams instead of a few grams (Linden, 1990). It was suggested that this difference could be due to both a morphological and a functional difference between the two types of endings (Hannam, 1976). Linden and Millar (1988b) suggested that it could be purely due to differences in the receptor sites since they observed a graded decrease in force threshold for receptors located between the fulcrum and the apex of a cat canine. Receptors with a higher force threshold were located closer to the fulcrum whereas receptors with a lower force threshold were located nearer to the apex.

In humans, it has been observed that the presence or absence of ear plugs affected force thresholds of periodontal mechanoreceptors, in that contributions from receptor sites other than those around the teeth may assist in the perception of a tooth tap in humans (Garton, 1969). It also has been found that the force thresholds of all receptors are influenced by the rate of force application (see below).

### ***Sensitivity to rate of force application***

It has been shown that periodontal mechanoreceptors are rate sensitive; when the rate of application of a given force on a tooth is increased, there is a corresponding rise in the frequency of impulse discharge (Hannam, 1969). It also was found that the rapidly adapting periodontal mechanoreceptors were more sensitive and slowly adapting ones were less sensitive in this respect. Moreover, the latency of the response of the rapidly adapting periodontal mechanoreceptor was shortened as the rate of application of the stimulus was increased.

The thresholds of all receptors were lowered when the rate of force application increased (Linden & Millar, 1988a; Loescher & Robinson, 1989a). The rate sensitivity of a particular receptor was found to be related to both the adaptation properties and the force threshold (Linden & Millar, 1988a). Together with the previous results (Linden & Millar, 1988b), it has been suggested that rate sensitivity also varies according to the position of the receptor, that the periodontal mechanoreceptors situated nearer to the fulcrum are more rate sensitive than those nearer to the apex. Similar observations on the rate sensitivity of the threshold level of periodontal receptors have been made in humans using a psychophysical approach (van Steenberghe & De Vries, 1978; Schoo *et al.*, 1983).

These findings suggest that different rates of application or velocities of mechanical stimulus applied to teeth may activate different periodontal mechanoreceptors. Therefore, this property needs to be considered when the effect of periodontal mechanoreceptors on the activity of jaw muscles is studied.

### ***Direction sensitivity and receptive fields***

There is strong evidence that periodontal mechanoreceptors respond maximally to a horizontal force applied in one direction in cats (Cash & Linden, 1982a; Karita & Tabata, 1985; Linden & Scott, 1989a); rabbits (Appenteng *et al.*, 1982) and humans (Johansson & Olsson, 1976; Trulsson *et al.*, 1992). When the stimulus is applied to a tooth in the most sensitive direction, which is specific to each periodontal mechanoreceptor, it evokes a larger response with a longer discharge time and a shorter latency in the initiation of the first impulse (Anderson *et al.*, 1970).

Furthermore, the threshold force is lowest if applied in this direction (Ness, 1954; Karita & Tabata, 1985). In humans, the directional sensitivity of mandibular periodontal mechanoreceptors was demonstrated by Trulsson *et al.* (1992) to be, that most afferent responses to static forces applied in two or three of the four horizontal directions and in two axial directions. In that study 61% of the units showed their strongest responses to forces in one of the horizontal directions, a majority were sensitive to the lingual or the labial direction, and in one of the axial directions, downward direction. It was demonstrated that even though individual periodontal mechanoreceptive afferents provide ambiguous information regarding the direction of a force applied to a tooth, populations of all afferents activated by applied force are well suited to give detailed directional information. It has been suggested that such information may play an important role in the control of mastication (Trulsson *et al.*, 1992).

The receptive fields of a periodontal receptor commonly extend beyond the individual tooth innervated (Anderson *et al.*, 1970), and may involve several teeth (Trulsson, 1993a). However, it has been suggested that this phenomenon is more likely to be caused by mechanical interaction between adjacent teeth (mechanical coupling) rather than axon branching (Trulsson, 1993a).

### ***Conduction velocities***

The conduction velocities of the afferent neurons that respond when a force is applied to the teeth have been measured in a number of studies (Pfaffmann, 1939; Hannam, 1968; Linden, 1978). These afferent neurons have conduction velocities that vary

between 26 - 87m/s with a mean of 54m/s (Linden, 1978). Hannam (1968) reported that velocities varies between 28 - 83 m/s, with most of the fibres conducting at about 50m/s. This is similar to the conduction velocity of A $\beta$  or group II fibres, which are involved in reception of touch and pressure in oral tissues (Linden, 1990).

### ***2.1.2 Central Projections***

The cell bodies of periodontal receptors are situated at two sites: the trigeminal ganglion (TG) and the trigeminal mesencephalic nucleus (MeV). The peripheral processes extend through the two divisions of the trigeminal nerve, whereas the central branches commonly divide in the brain to terminate in the main sensory nucleus and the spinal nucleus.

#### ***Trigeminal ganglion***

The TG is situated in the dural recess in the petrous part of the temporal bone, ventral to the pons. Responses from primary afferent neurons that respond to forces applied to teeth have been recorded using microelectrodes (Beaudreau & Jerge, 1968; Appenteng *et al.*, 1982; Cash & Linden, 1982b; Linden & Scott, 1989a). Linden and Scott (1989a) found that a large number of the periodontal mechanoreceptors with cell bodies in the TG were slowly adapting receptors and that they did not adapt to the prolonged force applied to the crown of the tooth.

#### ***Mesencephalic nucleus***

The MeV extends from the rostral end of the principal nucleus to the superior colliculus of the midbrain. Degeneration studies undertaken by Corbin (1940) and

Szentagothai (1948) have shown that many of the fibres in both the mandibular and maxillary divisions of the trigeminal nerve have their cell bodies in the MeV, and that among these are fibres supplying muscle spindles of the jaw closing muscles.

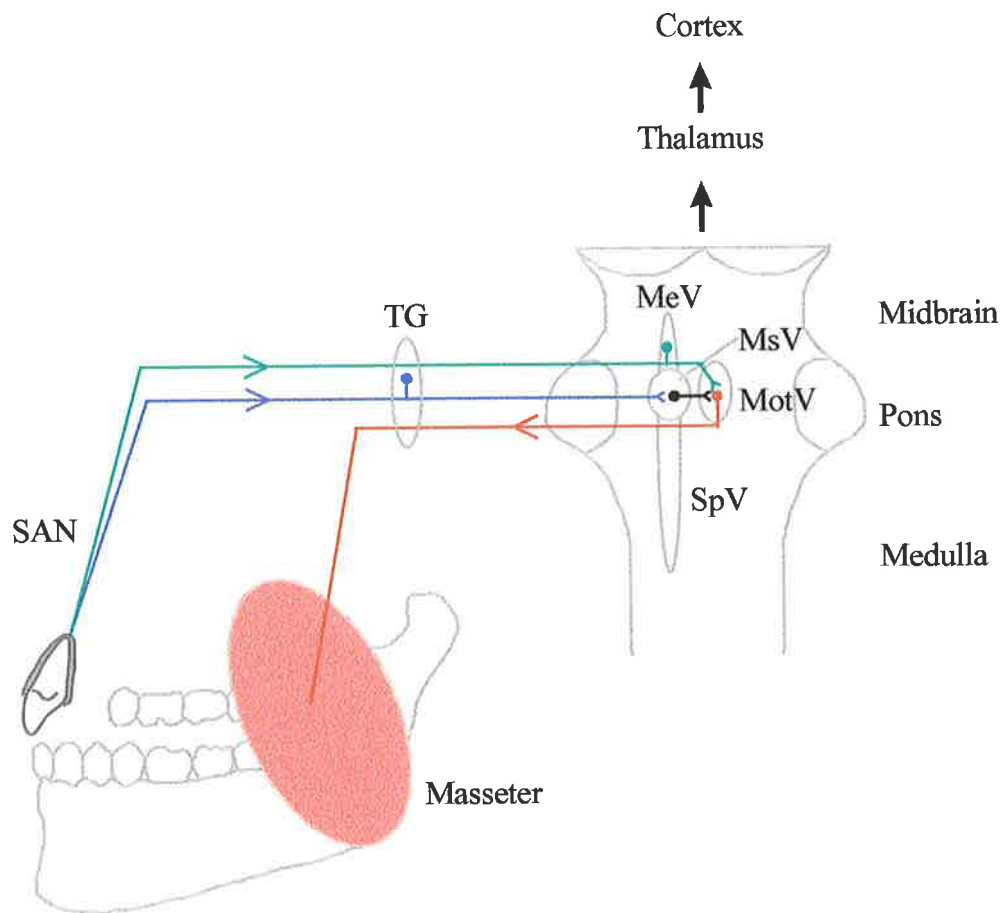
Unlike the TG, there is no evidence for slowly adapting periodontal mechanoreceptors having their cell bodies in the MeV (Jerge, 1963a; Linden, 1978; Lewis *et al.*, 1978). Byers *et al.* (1986) injected tritiated proline into the MeV of cats and found that all labelled MeV terminals in the EM autoradiograms were unencapsulated Ruffini-like mechanoreceptors, but they did not include as many large, complex endings as were found for TG receptors in rat periodontal ligament (Byers, 1985). Since the MeV neurons of periodontal mechanoreceptors have been shown to be less sensitive (higher threshold) and more rapidly adapting than TG neurons (Jerge, 1963a; Beaudreau & Jerge, 1968), it suggests that the large and complex endings may contribute to higher sensitivity and slower adaptation. It is of interest to speculate on the functional significance of having two distinct cell body populations. A correlative morphological study was carried out recently on two electrophysiologically identified and ink located periodontal ligament mechanoreceptors in anaesthetised cats. One mechanoreceptor had its cell body in the MeV and the other had its cell body in the TG. Both receptors had similar positions relative to the tooth apex and fulcrum. Only Ruffini nerve endings were observed under each ink marks. There was no apparent morphological difference between the two periodontal ligament mechanoreceptors (Linden *et al.*, 1994). This finding is not consistent with previously published results of these authors, which MeV receptors were found to be located in the apical part of the periodontal

ligament (Linden & Scott, 1989a). This discrepancy may be due to a very limited number of receptors (only two) being recorded in the later study.

### ***Interneurons***

The central projections of the cells in the TG send branches to the main sensory nucleus (MsV) and to the spinal nucleus of the trigeminal nerves (SpV). These nuclei form part of the trigeminal sensory complex and are located in the pons, medulla and spinal cord to the upper cervical level. It has been suggested that the pathways forming the intraoral mechanoreceptors to the cortex include two relays. One via the trigeminal brain stem nuclei (Figure 2.3) and the other via the ventrobasal thalamic nuclei (Lund & Sessle, 1974; Linden, 1990).

The central projections of the cells in the MeV are not well defined electrophysiologically. It has been assumed that they also send branches to the MsV and SpV, although the possibility that they excite the neurons in the motor nucleus (MotV) directly in a similar manner to the muscle spindles has also been suggested (Sessle & Schmitt, 1972).



**Figure 2.3 The central projection of periodontal mechanoreceptors**

Diagrammatic illustration of tooth, sensory nerve, first order afferent neurons, the trigeminal brain stem nuclei, and the masseter muscle. SAN= superior alveolar nerve; TG= trigeminal ganglion; MeV= trigeminal mesencephalic nucleus; MsV= trigeminal main sensory nucleus; SpV= spinal trigeminal tract nucleus.

The morphology and the central connections of MeV that respond to forces applied to teeth have been determined in the cat (Shigenaga *et al.*, 1988a,1989). Labelled axons and their collaterals terminated in the MotV, the SpV, the supratrigeminal nucleus (SupV), the intertrigeminal (IntV) and juxtatrigenial regions (Shigenaga *et al.*, 1988a). In a more extensive study of the cat (Shigenaga *et al.*, 1989), primary afferent neurons in the MeV were classified as slowly adapting or rapidly adapting neurons. This was according to their adaptation rate to forces applied to a canine tooth. The central connections of both neurons differed in their projections to the MsV, oralis nucleus of SpV, juxtatrigenial nucleus and MotV (Shigenaga *et al.*, 1989). The central axons of one neuron were suggested to travel caudally within Probst's tract and send collateral processes to other regions of the trigeminal complex. The other neuron terminated in the MotV but also gave off collaterals to the SupV and the IntV (Shigenaga *et al.*, 1988a).

Jerge (1963b) considered that the dorsomedial extension of the MsV is a separate entity (the nucleus supratrigeminalis). Unit activity was recorded in this region, and it was considered that this demonstrated interneuronal connections involving periodontal, palatal, and jaw muscle afferents.

### **2.1.3 Functional Roles**

The periodontal afferents have been attributed an important role in the sensorimotor regulation of chewing (Lavigne *et al.*, 1987; Inoue *et al.*, 1989; Morimoto *et al.*, 1989). There is some evidence to indicate that periodontal mechanoreceptors are



involved in the reflex control of the bite force (Kennet & Linden, 1987; Lund, 1991) and jaw muscle activities (Ottenhoff *et al.*, 1992a,b; Abbink *et al.*, 1998).

The control of small and steady forces was disrupted during periodontal anaesthesia indicating that periodontal mechanoreceptors can also contribute to the manipulation of a small holding force when an object is held between the teeth (Trulsson & Johansson, 1996). Subjects chose small and steady forces to achieve a stable bite, but they avoided higher forces at which the sensitivity of most periodontal mechanoreceptors was lower.

It also has been reported that periodontal afferent input is not necessary for the execution of a split task (Trulsson & Johansson, 1996). During a split task, the load on a tooth quickly reaches a magnitude at which the force-encoding capacity of most human periodontal mechanoreceptors' afferents saturate. They do not provide much information about the forces during a split task (Trulsson & Johansson, 1994,1995). However, there are some periodontal mechanoreceptor afferents that follow the force profile during the split phase rather well (Trulsson & Johansson, 1994). These afferent signals may play a role in providing a feedback to the jaw closing muscles during high levels of voluntary biting (Lund & Lamarre, 1973) and in the slow closing, 'power phase' of the chewing cycle (Lavigne *et al.*, 1987; Inoue *et al.*, 1989; Morimoto *et al.*, 1989).

Afferent information from these receptors also contribute to the spatial control of jaw actions. During anaesthesia of the periodontium, the subjects had difficulty in spatially

controlling the jaw, since necessary spatial information about the pattern of contacts across the dentition was not available for precise feedback and control of direction (Trulsson & Johansson, 1996).

There is strong evidence that periodontal mechanoreceptors play a role in the salivation reflex which occurs on chewing food (Kerr, 1961; Anderson *et al.*, 1985). Furthermore, there is a relationship between masticatory force and the salivary flow. Hector and Linden (1987) focused more precisely on the role of periodontal mechanoreceptors in the salivation reflex in humans. The results supported the hypothesis that intraoral mechanoreceptors and particularly periodontal mechanoreceptors contribute to the masticatory-salivary reflex.

## 2.2 JAW REFLEXES

The periodontal mechanoreceptor afferents have been attributed an important role in the sensorimotor regulation of chewing (Lavigne *et al.*, 1987; Inoue *et al.*, 1989; Morimoto *et al.*, 1989). Evidence that these receptors are involved in the reflex control of jaw muscle activity has been demonstrated in many studies (Matthews, 1975; Lund, 1991). Reflex responses of skeletal muscles have been studied in order to determine the synaptic relationship of afferents to motoneurons. Studying this relationship assists in the understanding of normal operation of the motor system, but also the abnormalities and their causes.

### ***2.2.1 Jaw Opening Reflex***

Stimulation of high threshold afferents reflexly excites jaw opening muscles and inhibits the background activity of the jaw closing muscles. This reflex was termed the jaw opening reflex. Sherrington (1917) observations showed that a jaw opening reflex could be evoked in decerebrate cats by blunt pressure applied to the gingival margins of the teeth, to the teeth themselves and to the anterior part of the palate. The jaw opening was shown to be due to both contraction of the jaw opening muscles and simultaneous inhibition of tonic jaw closing muscle activity (Matthews, 1975).

#### ***Excitation of jaw opening muscles***

In anaesthetised and decerebrated animals, the EMG activity recorded from digastric in response to electrical stimulation of the mucous membrane, periodontal tissues or infra-orbital nerve has shown an excitatory response with a latency of approximately 9 ms. Latency as short as 2.8 ms has been reported for the responses recorded from the nerve to digastric following supramaximal inferior dental nerve stimulation. From this finding it was suggested a disynaptic linkage exists (Matthews, 1975).

Periodontal mechanoreceptors have been shown to excite jaw opening motoneurons. Evidence comes from electrical stimulation of the periodontal ligament (Anderson & Mahan, 1971; Bailey & Capra, 1988), electrical stimulation of the alveolar nerves (Kidokoro *et al.*, 1968a) and the application of mechanical forces to the teeth (Hannam & Matthews, 1969; Sumino, 1971; Sessle, 1977).

Kidokoro et al. (1968a) and Sumino (1971) found that an excitatory post-synaptic potential (EPSP) and action potentials had been recorded intracellularly from digastric motoneurons following electrical stimulation of the inferior dental nerve in barbiturate-anaesthetised cats. This reflex response in the digastric motoneuron was attributed to the periodontal mechanoreceptors. However, Dessem and Taylor (1989) have similarly examined the reflex effects produced by graded-electrical stimulation of the inferior alveolar nerve in chloralose-anaesthetised cats. They found no digastric EMG activity as a result of stimulating the lowest-threshold fibres in this nerve. They concluded that these fibres were not capable, by themselves, of exciting digastric motoneurons.

In human studies, there is no evidence of activation of the digastric when teeth are stimulated (Matthews, 1975). Despite the absence of digastric activation, a small opening movement still occurs, presumably only due to the inhibition of jaw closing muscles activity (Türker & Miles, 1985).

### ***Inhibition of jaw closing muscles***

During the period of jaw opening reflex, the background activity in the jaw closing muscles is inhibited. The existence of an inhibitory reflex in the jaw closing muscles is known to occur (Sherrington, 1917). In this reflex, which is in some ways analogous to the withdrawal reflex in the limbs, a noxious stimulus leads to a reduction in the activity of the jaw closing muscles and an activation of the jaw opening muscles. A major function of this reflex is to reduce damage to the soft tissues in and around the mouth when a hard or sharp objects is inadvertently encountered. This inhibitory

reflex has attracted interest from clinicians, particularly since it has been claimed that the operation of the reflex is modified in patients suffering from facial pain syndromes, and that this may be used for the diagnosis and monitoring of the disease states.

Several studies have reported that the jaw closing muscles are reflexly inhibited as a results of normal tooth contact during mastication both in animals (Thomas & Peyton, 1983) and in humans (Ahlgren, 1969). This has prompted the use of mechanical stimulation as the means of investigating inhibitory jaw reflexes. By tapping an upper tooth, two silent periods in the jaw closing muscles EMG can be produced (Beaudreau *et al.*, 1969; Goldberg, 1971; Sessle & Schmitt, 1972). The latency and duration of the response depends on many variables but most observations indicate that the inhibition occurs at 10 - 13 ms (Brodin *et al.*, 1989,1993b).

It has been suggested that reflex jaw opening can be elicited by mechanical displacement of the teeth (Hannam & Matthews, 1969; Sessle & Greenwood, 1976; Thomas & Peyton, 1983) without exciting digastric motoneurons, since the threshold for inhibiting the firing of jaw closing motor unit is much lower that the threshold for exciting digastric EMG activity (Dessem & Taylor, 1989).

The evidence for reflex inhibition of jaw closing motoneurons from the periodontal mechanoreceptors has been reported by numerous investigators using a variety of techniques. Intracellular studies of jaw closing motoneurons have shown inhibitory postsynaptic potentials (IPSPs) during electrical stimulation of the alveolar nerves

(Kidokoro *et al.*, 1968a; Kawamura & Tanaka, 1971). Here it was shown that stimulation of the alveolar nerve produces two phases of hyperpolarisation in the jaw closing motoneurons with averaged latency of 9 ms and 40 ms. Since this IPSP can be evoked by electrical stimulation of the nerve, periodontal mechanoreceptors cannot be solely responsible for the IPSPs developed. Pulpal and cutaneous afferents which form the nerve also would have been activated by nerve stimulation (Cadden *et al.*, 1983). Stronger levels of stimulation increase hyperpolarisation and therefore indicates that activity of the smaller, higher-threshold fibres in this nerve also hyperpolarise the jaw closing motoneurons.

Further evidence for the inhibition of jaw closing motoneurons comes from studies on the firing of individual jaw closing motoneurons in response to electrical stimulation of alveolar nerve. Dessem and Taylor (1989) have recorded from a single motor unit (SMU) in the temporalis and the masseter muscles of cats and found that a marked inhibition of motor unit firing is produced following electrical stimulation of the inferior alveolar nerve. They also illustrated that inhibition of motor unit firing increases as the stimulus strength is increased. However, other afferent fibres as well as the afferents of periodontal mechanoreceptors may also have been activated by this type of electrical stimulation.

The most direct way to activate the periodontal mechanoreceptors is by displacing the teeth and in several animal studies this has been used to examine the reflex effects of periodontal afferents (Hannam & Matthews, 1969; Funakoshi & Amano, 1974; Sessle, 1977; Funakoshi, 1981). Sessle (1977) reported inhibition of jaw closing motoneuron

discharge following tooth-tapping in cats. An inhibition of jaw closing EMG activity following tooth-tapping has frequently been reported in humans (van Steenberghe, 1979). Dessem *et al.* (1988) examined the reflex effects of tooth displacement in chloralose-anaesthetised cats. The firing of SMUs in the masseter and temporalis muscles was recorded during mechanical displacement of the maxillary teeth and used to compute peristimulus time histograms (PSTHs). When 0.48 N of force was applied to the tooth, inhibition of the SMU was evident in the PSTHs and its cumulative sum (CUSUM) which displayed the cumulative sum of the deviation from the control mean of successive bins (Ellaway, 1978). As the magnitude of force is increased to 1 N, the inhibition became very marked. Two phases of inhibition could be seen in the PSTH with the averaged latency of approximately 15 ms and 33 ms. This corresponded to the latency reported in the masseteric response following stimulation of the inferior alveolar nerve (Kidokoro *et al.*, 1968a). When 2 N of force was applied to the tooth, the inhibition of motor unit firing was profound and the two phases of inhibition appeared fused (Dessem *et al.*, 1988).

Reflex responses of SMUs of the masseter and temporalis, to a tap applied to a maxillary tooth were studied in humans (Bonte & van Steenberghe, 1989). They found that a tap to a tooth has inhibitory effects on jaw closing motoneurons. During the inhibition, the SMU with small spike amplitude and high prestimulus firing frequency was reactivated before the SMU with large spike amplitude and low prestimulus firing frequency. In all reflex responses, evidence was obtained that the SMU spikes were reactivated after the inhibition in a clustered manner. The apparent excitation seen in

histograms was most likely due to the synchronised reactivation of the silenced SMU spikes (Miles *et al.*, 1987; Bonte & van Steenberghe, 1989).

Detailed knowledge concerning reflex pathways of the masseteric inhibitory reflex in humans is not yet available. Considering the location of the reflex paths underlying the two components of masseteric inhibitory reflex, the following from animal studies is worthy of mention. The early inhibitory reflex is mediated by one interneuron in the supratrigeminal nucleus. From this neuron, collaterals run either ipsilaterally before they terminate in the ipsilateral trigeminal motor nuclei or contralaterally before they terminate in the contralateral trigeminal motor nuclei (Kidokoro *et al.*, 1968a,b; Nakamura, 1980). With respect to the late inhibitory component, it has been demonstrated that stimulation of the bulbar reticular formation at the ponto-medullary junction exerts an inhibitory effect on trigeminal motoneurons. Furthermore, sectioning of the brain stem caudal to the trigeminal motor nuclei abolished the existence of the late inhibitory reflex (Goldberg, 1968; Nakamura, 1980). Inhibitory reflexes may not utilise secondary neurons of the trigeminal nucleus on both sides. Anatomically it has been demonstrated that interneurons projecting upon the trigeminal motoneurons lie in the medial propriobulbar system of the lateral reticular formation (Kidokoro *et al.*, 1968a).

It is not known whether the jaw opening reflex pathway is continuously activated in normal function or whether it only operates as a protective mechanism against trauma (Ongerboer de Visser & Cruccu, 1989). It has been proposed that jaw opening reflex



and jaw closing muscles are both important for the co-ordination of jaw movements in mastication (Nakamura, 1980).

### **2.2.2 Jaw Closing Reflex**

Sherrington (1948) demonstrated that the jaw opening reflex could be converted into a jaw closing reflex by strychnine or tetanus toxin, both of which are known to block inhibitory mechanisms. One interpretation of this observation would be that, in the absence of the drugs, both excitatory and more powerful inhibitory inputs were activated, but only the excitatory effect remained after the inhibition had been blocked (Matthews, 1975). This interpretation has been confirmed by the finding that, in anaesthetised cats, the first phase of inhibition of the jaw closing muscles reflex produced by inferior dental nerve stimulation is converted into a period of facilitation by an intravenous injection of strychnine (Sumino, 1971). These excitatory effects are usually masked by the simultaneous activation of inhibitory pathways.

There is also some evidence of reflex activation of jaw closing muscles in humans following electrical stimulation of the mucous membrane over the root of a tooth (Sessle *et al.*, 1973). For these neural connections from mucosal or periodontal mechanoreceptors, it can be assumed that the excitatory effects are normally opposed by inhibition from the same source, so that any tendency for positive feedback to occur is self-limiting and confined to a few milliseconds after tooth or mucous membrane stimulation. At present, only speculation on the possible functional significance of such a situation can be made.

Lund and Lamarre (1973) have shown in humans, that local anaesthesia of teeth greatly reduces the maximal voluntary biting force. On this basis, they proposed that periodontal mechanoreceptors add excitation to the jaw closing muscles during chewing in order to overcome the additional resistance resulting from objects between the teeth. Further evidence comes from animal studies. In rhythmically chewing anaesthetised rabbits, it has been shown that extra EMG activity is induced in the jaw closing muscles when a steel ball is placed between the teeth during stimulus-evoked chewing. This extra muscle activity was decreased when the input from the periodontal ligament receptors was removed (Lavigne *et al.*, 1987).

There is also evidence that periodontal mechanoreceptors can excite jaw closing motoneurons. In rats, pressure applied to the maxillary incisor teeth produced an early transient and a late tonic increase in the masseter muscle activity, both of which were lost if the superior alveolar nerve was sectioned (Funakoshi & Amano, 1974; Funakoshi, 1981). The early transient response was abolished by transections of the brain stem between the trigeminal motor nucleus and the mesencephalic nucleus. The tonic response was abolished by transections between the trigeminal motor nucleus and the trigeminal sensory complex nucleus, which contains trigeminal main sensory nucleus and trigeminal spinal tract nucleus. Initiation of the transient response depends on the rate of rise of the initial phase of mechanical stimulation to the tooth, while that of the tonic response was dependent on the intensity of the stimulation. A tonic increase in the firing of single masseter motoneurons has been reported to occur while pushing on the teeth in humans (Amano & Yoneda, 1980). This response was found in approximately one half of the motor units tested in the anterior part of the

masseter muscle and was dependent upon the maintenance of a weak voluntary isometric contraction. However, this type of facilitation of jaw closing muscle activity was not observed during tooth displacement in experiments of chloralose-anaesthetised cats (Dessem *et al.*, 1988) or human subjects (Louca *et al.*, 1994, 1996a).

It has been demonstrated that the rate of rise of tooth stimulation force determines the reflex response of jaw closing muscles. Where a push (slowly-rising) force was applied to an upper incisor tooth for the reflex study of jaw closing muscles (Brodin *et al.*, 1993b), it was found that the push stimulus evoked a long-latency, primarily excitatory response. However, with stronger stimuli, the short-latency inhibitory response often became evident before the onset of the excitation. Since the reflex responses to 3 N pushes were abolished when the receptors around the tooth were blocked with local anaesthetic, the reflex responses were initiated from the receptors located within the periodontal area (Brodin *et al.*, 1993b). Subsequently, the reflex responses evoked by controlled mechanical stimulation of a tooth in single motor units in the human masseter muscle were examined (Türker *et al.*, 1994). Here the reflex responses of the motor units were characterised by analysis of the changes in the duration of the first and second inter-spike intervals (ISIs) immediately following the stimulus. A significant decrease in the duration of these ISIs in comparison with prestimulus ISI indicated a reflex excitation in response to slow push stimulus to an upper incisor. It was concluded that periodontal mechanoreceptors can activate an excitatory reflex pathway to the jaw closing motoneurons (Türker *et al.*, 1994).

### 2.2.3 Effects of Periodontal Mechanoreceptors on Jaw Reflexes

There is little doubt that the forces produced during mastication and biting are sufficient to activate the periodontal mechanoreceptive afferents (Larson *et al.*, 1981; Appenteng *et al.*, 1982). Evidence that the afferent input from these mechanoreceptors is not blocked during jaw closing is provided by the work of Yamada and Shimada (1984), in which rhythmical jaw movement in lightly anaesthetised rats could only be maintained when incisal tooth contacts were present. Ottenhoff *et al.* (1992a,b) pointed out that muscle activity increases with increasing hardness of the food and therefore, periodontal mechanoreceptor feedback must be conveying information about the physical nature of the food (Thexton & McGarrick, 1987).

Several hypotheses have been put forward for the role of the periodontal mechanoreceptors in the control of jaw muscle activities during mastication and biting. Sherrington (1917) proposed that when a piece of food came into contact with gums, teeth or hard palate during jaw closure, a jaw opening reflex was elicited, coupled with rebound jaw-closure producing a rhythmical masticatory pattern. It has been generally known that rhythmical jaw-muscle motoneuron firing is produced by the central pattern generator (Dellow & Lund, 1971) and manipulated by the peripheral sensory feedback (Appenteng *et al.*, 1982; Inoue *et al.*, 1989; Morimoto *et al.*, 1989). Since the discharge of the periodontal mechanoreceptors is related to the magnitude and direction of force applied to the teeth, those receptors are well situated to provide sensory feedback during the occlusal phases of mastication and biting.

Several researchers have proposed a feedback role for these receptors and their hypotheses can be categorised into 3 types:

- a negative feedback role (Sessle & Schmitt, 1972; Sumino, 1976; Van Der Glas *et al.*, 1985; Dessem *et al.*, 1988; Bonte *et al.*, 1993; Louca *et al.*, 1994, 1996a);
- a positive feedback role (Lund & Lamarre, 1973; Amano & Yoneda, 1980; Funakoshi, 1981; Lavigne *et al.*, 1987; Inoue *et al.*, 1989; Morimoto *et al.*, 1989);
- a combination of negative and positive feedback, depending upon the magnitude of force applied to the mechanoreceptors (Thexton, 1973, 1974; Luschei & Goldberg, 1981; Brodin *et al.*, 1993b; Türker *et al.*, 1994).

Taylor and Gottlieb (1985) proposed that jaw movement was controlled by negative length feedback from muscle spindles during jaw closing. When the jaw encounters a resistant food item, the control is switched to a force servo in which the periodontal receptors provide a negative feedback of force. Since the median threshold for inhibitory response was 0.25 N and this was significantly less than that for the subsequent excitatory waves (median: 0.75 N), it was suggested that ramped mechanical stimulation of teeth may preferentially produce reflex inhibitions rather than reflex excitations (Louca *et al.*, 1996a). It seems unlikely that a simple negative force feedback can provide the increases in jaw closing EMG activity when a resistant food item is encountered (Abbink *et al.*, 1998).

Lund and Lamarre (1973) proposed that the periodontal mechanoreceptors provided positive feedback to the jaw closing muscles based upon a reduction in maximum voluntary bite force in human subjects following the infiltration of local anaesthetic around the teeth. This possibility is further supported by the increases seen in the

EMG activity of jaw closing muscles when an obstruction is placed between the molar teeth of a rabbit during cortically induced jaw closing movements (Lavigne *et al.*, 1987; Inoue *et al.*, 1989; Morimoto *et al.*, 1989). If these afferents only provide positive feedback during jaw closing, then some other mechanism must exist to break the positive feedback loop and limit the amount of bite force produced.

Thexton (1973,1974) has proposed that a sequence of reflex responses is elicited depending upon the magnitude of force applied to the palatal mechanoreceptors. Luschei and Goldberg (1981) have suggested that both excitatory and inhibitory effects from the periodontal mechanoreceptors are utilised in controlling bite force. They suggest that one class of mechanoreceptors (presumably low threshold) provide positive feedback to the jaw closing muscles and therefore increase the biting force. As the bite force reaches a certain magnitude, other mechanoreceptors with higher thresholds or sensitivity to sustained pressure would be activated and limit the bite force. However, tooth displacement studies provide little evidence for excitation of jaw closing muscles even using very small stimulation force (Dessem *et al.*, 1988). The reflex response evoked in the human masseter in response to varying rate of rise of mechanical stimulation was examined electromyographically (Brodin *et al.*, 1993b). With rapidly-rising tap force, a short-latency inhibitory reflex was elicited in the masseter EMG activity. With slowly-rising push force, a long-latency primarily excitatory response was demonstrated in the masseter EMG. This finding suggests that the rate of rise of applied force initiated different reflex effects in the jaw closing muscles (Brodin *et al.*, 1993b). With a similar stimulation paradigm, Louca *et al.* (1994,1996a) have shown that the only significant responses produced by the ramp

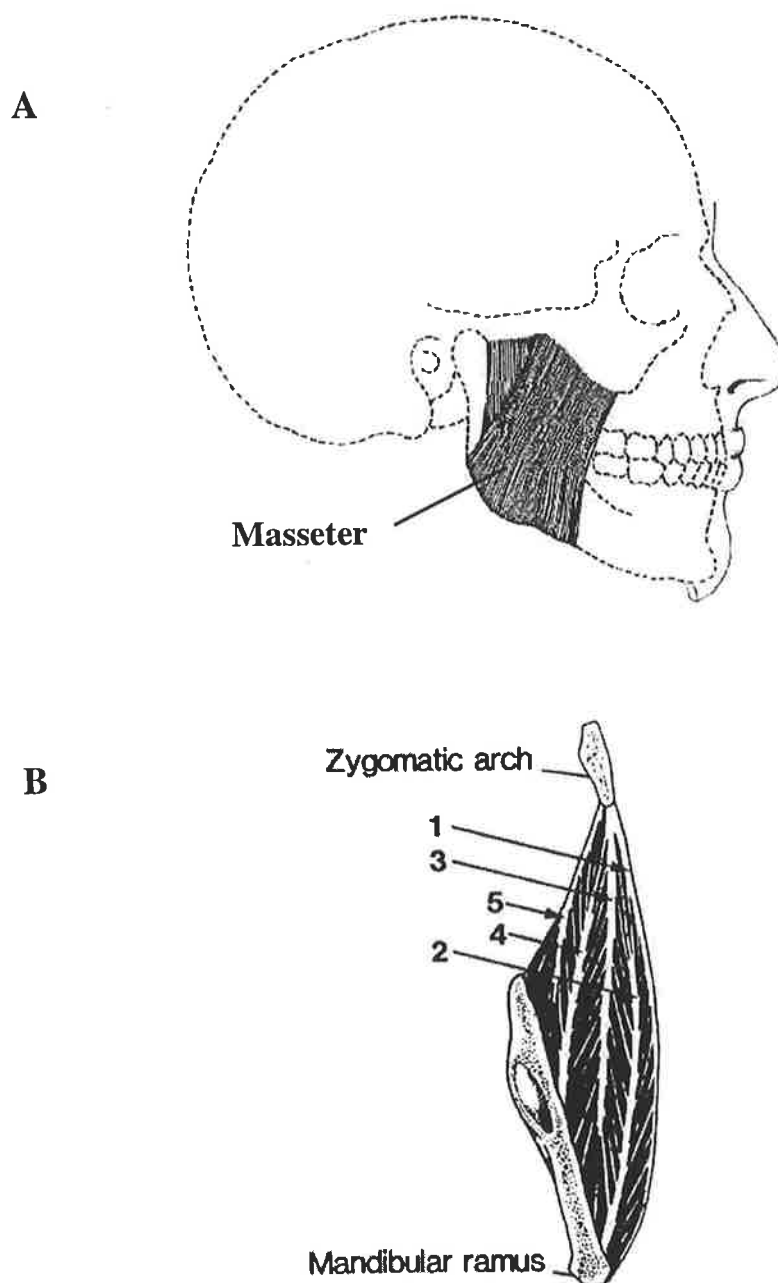
stimuli were short latency inhibitory reflexes in the human masseter. To explain these contradictory findings, the hypothesis has been put forward that some other factors may influence the reflex outcome in jaw muscle.

## 2.3 MASSETER

The jaw closing muscles contribute to the posture of the mandible and forces used for chewing. Since the masseter muscle is the most powerful of the muscles for mastication, studies have been focused on this muscle. The masseter muscle has its origins in the zygomatic arch and it terminates at the angle of mandible (Figure 2.4). When the muscle contracts, it elevates the mandible, closing the mouth, and also producing bite force (Brand & Isselhard, 1994).

### 2.3.1 Structure

Topographical descriptions of the adult human masseter can be found in numerous publications (Schumacher, 1961; Miller, 1991; Williams *et al.*, 1995). The masseter has been described to comprise three parts - superficial, intermediate, and deep. 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 into 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 into the central part of the ascending ramus. A deep part arises from the deep surface of the zygomatic arch and inserts into the upper part of the ascending ramus (Hannam & McMillan, 1994).



**Figure 2.4 Diagrammatic illustration of the human masseter**

**A.** A diagram of the masseter, adapted from *The Anatomy of Oral-Facial Structures*, 5<sup>th</sup> (1994). Edited by Brand, R.W. and Isselhard, D.E.

**B.** Longitudinal section from a frontal view of the masseter, adapted from Stålberg and Eriksson (1987). Muscle fibres are black, tendinous tissues are white. Note five tendon plates (marked by numbered arrows) running in parallel from the zygomatic arch and the mandibular ramus.



The internal structure of the masseter is complex (Stålberg & Eriksson, 1987). It consists of five internal aponeuroses that run parasagittally and attach to the zygomatic arch and mandible (Figure 2.4). The deep, intermediate and superficial parts of the 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). The 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 & McMillan, 1994; Weijs & Kwa, 1995). The length of the muscle fibres ranges from 14 mm to 38 mm, with the longest fibres located anteriorly (Van Eijden & Raadsheer, 1992). Most of the masseter 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 region, the fibres are more horizontal, converging from the mandibular angle to the anterior end of the zygomatic arch. The deep region fibres are more vertically oriented (Hannam & McMillan, 1994; Weijs & Kwa, 1995)

Information about regional differences in fibre, tendon and sarcomere lengths has been provided recently in a detailed study by Van Eijden and Raadsheer (1992). These differences in regional morphology imply functional differentiation.

### 2.3.2 Motor Units and Muscle Fibres

#### *Motor unit*

The cell bodies of the motor efferent fibres, which lead to the skeletal muscle fibres, are called *motoneurons* (Schmidt, 1985). The group of motoneurons innervating an entire muscle is referred to as the *motoneuron 'pool'* for that muscle. Each motoneuron of a pool innervates a number of muscle fibres of the corresponding muscle to form the *motor unit* (Calancie & Bawa, 1990). Thus, a motor unit includes the entire cell body and dendrites of the motoneuron, the axon, and the muscle fibres innervated by the axon (Stuart & Enoka, 1983).

It is now known from the study of limb muscles that as a functional unit, motor units have some basic characteristics which are summarised as follows:

- All muscle fibres of a motor unit lie within a single anatomically-defined muscle (Stuart & Enoka, 1983).
- There is a one-to-one relationship between the discharge of a motoneuron and all the muscle fibres of the motor unit (Bigland-Ritchie *et al.*, 1979).
- Each muscle fibre is innervated by only one motoneuron (Burke, 1981).
- All the muscle fibres within the motor unit have a very similar metabolic profile (Burke *et al.*, 1971, 1973; Nemeth *et al.*, 1986).

With the basic characteristics of the motor unit, study of muscle function at the level of the motor units has contributed to the understanding of how muscles perform their tasks.

### ***Motor unit types***

Differences in classification of units have arisen because different researchers have generally concentrated either on histochemical properties of the muscle fibres of which they are comprised, or on physiological features of the motoneurons supplying them.

Considering the physiological properties, motor units have been classified into two groups, slow contracting and fast contracting. The slow-contracting units (Type-S) are very resistant to fatigue, and the fast contracting population can be separated into three groups with varying resistance to fatigue: Type-FF for fast-contracting highly fatigable units; Type-F(Int.) for fast-contracting units of intermediate fatigability; Type-FR for fast-contracting units of low fatigability (Burke *et al.*, 1973). Type-FF motor units produce much more force output than Type-S motor units because the innervation ratio (the number of muscle fibres in the motor unit) is higher, and each of the muscle fibres generate higher forces due to their greater cross-sectional areas (Ghez, 1991). Type-S motor units usually have the lowest recruitment thresholds whereas type FF motor units are recruited at higher force levels (Eriksson, 1982).

Based on histochemical reactions, the muscle fibres have been divided into two groups, Type-I and Type-II (Dubowitz & Pearse, 1960). It has been shown that large scale myosin ATPase (MATPase) activity correlated well with contraction speed (Barnard *et al.*, 1971) and single unit studies have confirmed that Type-I units correspond to the slow contracting type in normal cat muscles (Burke *et al.*, 1971; Taylor, 1976). Brooke and Kaiser (1970) showed that the Type-II fibres in human

muscle also contained the same subgroups (Type-IIA, IIB, and IIC) found in animal muscles (Brooke & Kaiser, 1970). The combination of physiological and histochemical tests has become a standard for categorising motor unit populations in subsequent studies. Evidence suggests that the Type-S motor units contain the Type-I muscle fibres, FF have Type-IIB, FR have Type-IIA and F(Int) have Type-IIC muscle fibres which stain moderately for oxidative enzymes, and have moderate to high tetanic tensions (for review: Stuart and Enoka, 1983).

Muscles in the body differ in the relative proportions of the fibre types. In general, most muscles have a mean fibre composition of about 50% Type-I fibres, but some muscles such as soleus may have close to 100% Type-I fibres in some individuals, while triceps brachii may have less than 20% Type-I (Johnson *et al.*, 1973). Type-II muscle fibres are larger in diameter than the Type-I fibres, except those in jaw muscles (Eriksson, 1982; Eriksson & Thornell, 1983; Eriksson *et al.*, 1984).

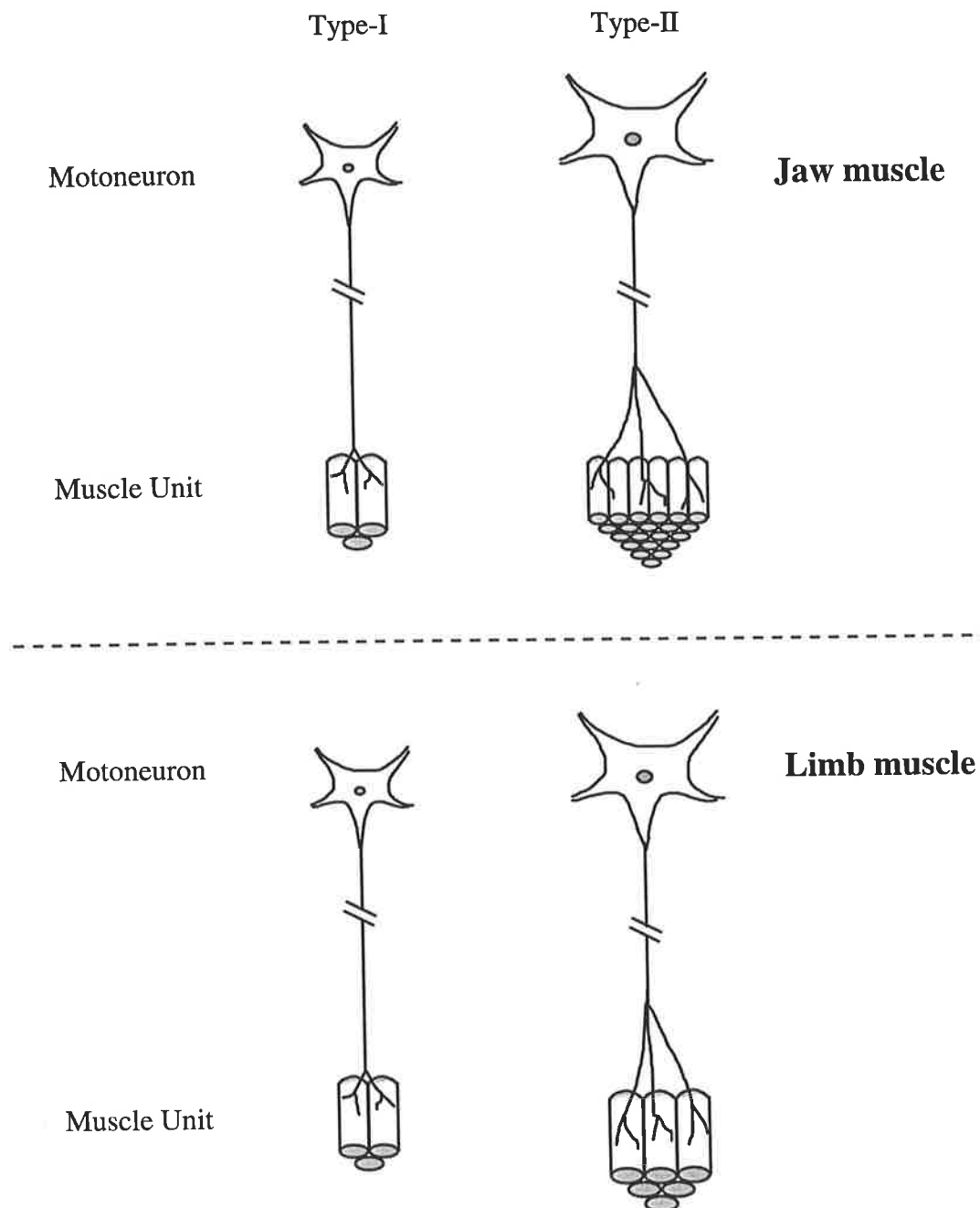
The motoneurons supplying the motor units demonstrate characteristics supportive of the function of the motor units. Type-S motor units have small, high resistance motoneurons with slowly conducting axons. Type-FF motor units have large, low resistance motoneurons with rapidly conducting axons. Thus, 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 (Munson, 1990).

### ***Masseter motor units***

The histochemical properties of jaw muscle fibres differ between species (Luschei & Goldberg, 1981; Miller, 1991; Mao *et al.*, 1992). Evidence from a number of studies indicate that the histochemical profile of the masseter differs from that in limb muscles.

The main differences are summarised below:

- The diameter of Type-I fibres is greater than those of Type-II fibres (Eriksson & Thornell, 1983). This contrasts strongly with the limb and trunk muscles, where Type-II fibre diameter is greater than Type-I fibre diameter (Burke, 1981)(Figure 2.5). This may be due to the lack of exercise in the masseter, due to the soft refined foods in the modern diet (Eriksson & Thornell, 1983).
- A large proportion of fibres with intermediate staining for MATPase (MATPase-IM) and type-IIC fibres are found in the jaw muscles (Eriksson & Thornell, 1983). Fibres of this type are rare or non-existent in normal adult limb muscles (Dubowitz & Brooke, 1973). It was suggested by Eriksson and Thornell (1983) that the existence of this population of fibres may explain the continuous distribution of motor unit contraction times in the human masseter (Yemm, 1977; Goldberg & Derfler, 1977).
- Masseter muscles in general rarely contain type-IIA fibres (Ringquist *et al.*, 1982; Eriksson & Thornell, 1983). Type-IIA fibres correspond to motor units of the Type-FR, and it has been suggested that the finding of limited Type-IIA fibres is possibly due to adaptation to functional demands (Eriksson & Thornell, 1983).



**Figure 2.5 Motor unit of human jaw muscle and limb muscle**

Type-I and Type-II muscle units and their corresponding motoneurons of human jaw and limb muscles are illustrated. Note that the diameter of Type-I fibre is greater than the fibre of Type-II in jaw muscle. However, in limb muscle, the fibre diameter is correlated to histochemical type so that Type-II fibre diameter is greater than Type-I diameter.

- Histochemical methods also reveal that the masticatory muscles do not have the mosaic pattern of fibre type distribution normally found in the limb muscles, but rather have large groups of densely-packed fibres of the same histochemical type (Dubowitz & Brooke, 1973; Eriksson & Thornell, 1983). Such an appearance in skeletal muscle would be considered pathological (Dubowitz & Brooke, 1973), but it is apparently normal in the masseter (Schwartz *et al.*, 1976).

There are other differences between jaw and limb muscles. Type-I fibres are predominant in almost all parts of the adult human masseter, where they make up 62-72% of the muscle's cross-sectional area (Eriksson & Thornell, 1983). In contrast, Type-II fibres occupy only 7-12% of the cross-sectional area. The Type-I fibres in the masseter contain slow myosin, and its Type-II fibres contain mainly fast myosin, similar to the limb muscles (Thornell *et al.*, 1984; Hannam & McMillan, 1994). In the anterior portion (deep and superficial layers), Type-I fibres account for about 70% of all fibres (Eriksson, 1982). This suggests that the anterior masseter is highly resistant to fatigue, and that most units will be activated at low forces, allowing more precise control over masticatory forces (Mao *et al.*, 1992). The posterior superficial masseter has the highest percentage of Type-II fibres, suggesting a capacity in this part of the muscle for higher muscular tension but with fatigue occurring more quickly. However, Tuxen *et al.* (1999) found that in the deep superficial part of the anterior masseter, Type-II fibres predominated, both in cross-sectional area and number. Most of these were Type-IIA (Tuxen *et al.*, 1999). They explained that the differences in biopsy

location and the subject's morphological uniqueness might be the cause of this contradictory result.

From low-threshold units, Stålberg *et al.* (1986) estimated that small units would contain about 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 in the number of muscle fibres per unit. Considering the innervation ratio differences in Type-I and II motor units in the masseter, the cross-sectional area of Type-II motor units are still larger than Type-I motor units (Figure 2.5), since in the masseter the diameter of Type-I muscle fibre are only 1-2 times larger than the Type-II fibre's (Stålberg *et al.*, 1986).

In contrast to other muscles, there is no animal data regarding the physiological properties of individual motor units in the masseter. This is presumably because of the difficulty in gaining access to the motor nerves without damaging the muscle. Consequently, the physiological properties of motor units in the jaw muscles of animals have mostly been inferred from whole-muscle studies. As the masseter is primarily composed of Type-I fibres, it would be expected that there would be a large number of Type-S motor units. However, the consensus of the few animal studies which have appeared in the literature is that the jaw muscles are fast-twitch muscles. Fast contraction times have been reported in the masseter of the rat (Nordström & Yemm, 1974) and of the possum (Thexton & Hiimae, 1975). The human masseter also appears to behave as a fast muscle (Matthews, 1996), with high levels of fatigue and a poor correlation of motor fatigability with motor unit twitch amplitude and contractile speed, as measured by spike-triggered averaging (STA) of force with the



firing of a motor unit (Nordstrom & Miles, 1990). This led them to conclude that the physiological properties of human masseter motor units are in fact poorly correlated with histochemical type. In the study by Yemm (1977) using STA technique, the masseter units had a continuous range of contraction times from 24 ms - 91 ms, with no evidence for separate populations of fast and slow motor units. There also was no correlation between twitch tension and contractile speed of the units; in fact in some subjects there was a significant positive correlation, which is opposite to the pattern reported in human first dorsal interosseous (Milner-Brown *et al.*, 1973c) and to the normal pattern seen in the animal muscles studied (Burke, 1981). In the study by Goldberg and Derfler (1977) the population of units examined was generally of higher recruitment threshold than the units in Yemm's study. It also was found there was a continuous distribution of contractile speeds in the motor unit twitches, that were distributed over a narrower range (36 ms - 69 ms), and they also found no correlation between twitch tension and contractile speed.

In summary, Type-I fibres clearly predominate in most human masseter muscles, especially in the anterior and deeper regions. Physiological studies, however, contradict these findings. Fibre-type grouping is apparently normal in these muscles and would be considered pathological if it occurred in the limb or trunk muscles, where it would indicate denervation and reinnervation. Since motor units comprise fibres of one histochemical type, it is possible that masseter motor units are confined to regions that are defined histochemically, rather than distributed randomly throughout the muscle.

### 2.3.3 Neuromuscular Compartments

Task-dependent, differential activation of superficial and deep masseter fibres have often been reported (Belser & Hannam, 1986; Tonndorf & Hannam, 1994), but it now seems that functional partitioning of activity is possible in at least three regions of the masseter muscle; deep anterior, deep posterior, and superficial. In humans, heterogeneous behaviour is obvious during biting efforts in different directions (Blanksma *et al.*, 1992). Masseter fibres are capable of some degree of selective activation when the occasion demands it. The distribution of tension across the muscle is not uniform, and the magnitude and orientation of muscle force vectors will alter according to function (Van Eijden & Raadsheer, 1992).

In the pig, masseter motor unit territories revealed by glycogen depletion methods (Herring *et al.*, 1989), showed that the motor units are mainly contained within individual fascicle boundaries. In contrast, limb muscle motor unit territories are not generally restricted to fascicle boundaries. If most groups of fibres are arranged in discrete functional territories related to muscle architecture with others occupying larger zones, the nervous system would need to balance patterns of motor unit recruitment with the activation thresholds, territorial sizes, and locations of the motor units available. Thus, both the size and location of motor unit territories are fundamental to hypotheses regarding the control of jaw muscle function (Hannam & McMillan, 1994).

Primary branches supply discrete subvolumes of muscle, termed neuromuscular compartments or partitions (English & Weeks, 1984; Windhorst *et al.*, 1989). It has

been postulated that compartmentalisation provides a peripheral anatomical substrate for motor control (English & Weeks, 1984). Within neuromuscular compartments of the pig masseter, fibre types are relatively homogeneous, but there are marked differences between compartments (Herring *et al.*, 1979). Experiments involving stimulation of individual axons of the pig masseter have confirmed that motor units are confined to single neuromuscular compartments (Herring *et al.*, 1989). There are at least three primary nerve branches in the human masseter (Schumacher, 1989). They supply its anterior, inferolateral, and deep regions, which might be considered neuromuscular compartments.

Neuromuscular partitioning in the jaw muscles should be considered with due regard for two other physiological concepts of muscle subdivision during function. These are sensory partitioning, which refers to the preferential response of muscle sensory afferent fibres to localised mechanical perturbations within the muscle, and central partitioning, which refers to the regionalisation of segmental connections within each respective motor nucleus.

In summary, the localisation of motor units in neuromuscular compartments offers the potential for more specific motor control than contraction of the whole muscle. It would permit the activation of muscle regions relatively independently of one another, with the regional interactions contingent on the particular task and movement strategy. There is good anatomical support for the likelihood of sensory (reflex) partitioning in the human masseter, therefore, this information should be considered during reflex studies.

## 2.4 FORCE DEVELOPMENT

The production of force by a muscle is achieved by activation of the functional unit of muscle, the motor unit. Control over the level of force produced during a contraction is achieved by an increase or decrease of the number of active motor units (recruitment or de-recruitment). Once a motor unit is recruited, further control in its force output can be achieved by variation of its firing rate (Calancie & Bawa, 1990).

### 2.4.1 *Recruitment and the Size Principle*

In most voluntary and reflex contractions studied to date, motor units have been found to be recruited into activity in an orderly sequence rather than a random fashion. This phenomenon is known as *orderly recruitment* (Stuart & Enoka, 1983). The orderly nature of motor unit recruitment was first recognised by Denny-Brown and Pennybacker (1938), who observed a consistent and reproducible recruitment order for motor units in voluntary contractions.

Extensive experimental evidence for a relatively fixed recruitment and de-recruitment order based on the size of the motor unit has been provided by Henneman (1957), and has come to be known as the 'size principle' of motor unit recruitment, or normal recruitment order (Henneman & Mendell, 1981). Evidence for a size-structured motor unit recruitment hierarchy came from study of the decerebrated cat (Henneman & Olson, 1965; Henneman *et al.*, 1965b), in which it was demonstrated that size-related motor unit recruitment was very reproducible and independent of the source of input. In a later study with the decerebrate cat it was shown that motor units exhibited a critical level, so that all units responded reliably to input drive above their critical

level, and failed to respond to input drive below their critical level, or functional threshold (Henneman *et al.*, 1974). In Henneman's Law of Combination, at any level of net excitatory drive, the last-recruited unit is the largest active unit, and all smaller units continue to discharge as the drive is increased (Henneman *et al.*, 1974). The essence of the size principle of motor control advanced by Henneman is that motoneurons within a motoneuron pool receive qualitatively identical inputs (Henneman *et al.*, 1965a), with the excitability of each motoneuron closely related to its input resistance, which varies inversely with cell size (Henneman & Mendell, 1981). The functional significance of the size principle, in which smaller motoneurons within a pool show higher susceptibility to discharge than larger motoneurons, is of importance for the control of force production by the central nervous system (CNS).

Muscle units innervated by small motoneurons tend to produce small twitches and tetanic forces but are highly resistant to fatigue (Burke, 1967). Conversely, large motoneurons tend to produce large twitches and tetanic tensions but are easily fatigued. The orderly recruitment of small before large motoneurons would thus allow for smooth increments of force at any level of contraction (Harrison, 1983). Secondly, movements or behaviours requiring only small forces could be continued for long periods with little or no fatigue (Calancie & Bawa, 1990). Furthermore, such an arrangement contains the possible combinations of units to perform a task into consistent subsets, and greatly simplifies the amount of central processing needed for motor control (Henneman *et al.*, 1965b).

As the size of each motor unit is closely related to that of its parent motoneuron, motor unit recruitment is conventionally believed to be based on the 'size principle' (Henneman & Mendell, 1981). Throughout the last 30 years, the validity of the size principle in animals and humans has been tested through the examination of motor unit recruitment and derecruitment patterns produced by various inputs to motoneuron pools. Voluntary contractions have been mainly tested in human subjects which provide a more extensive and subtle range of behaviours to animals. The first demonstration of orderly recruitment of motor units in humans, used the spike-triggered averaging technique (STA, Stein *et al.*, 1972) to extract the average-twitch waveform produced by the contraction of single motor units of the first dorsal interosseous muscle from the force fluctuations of the whole muscle. This technique was also used in other studies to study the mechanical properties of human motor units activated voluntarily in human first dorsal interosseous muscle (Milner-Brown *et al.*, 1973a,b,c). A unimodal distribution of motor unit contraction times (30 ms - 105 ms) along with a negative correlation between twitch tension and contractile speed was reported. An important finding was that the motor units were recruited in order of increasing twitch tension during a voluntary contraction, which further supported the size principle of recruitment. The deficiency of the STA technique is that it tends to underestimate twitch contractile speed due to partial fusion of the twitches (see Methodological Considerations).

By using different indicators of motor unit size (twitch amplitude, contraction time, recruitment threshold, and conduction velocity of motor axon or muscle fibre), orderly recruitment of motor units during slow-ramp contractions has been reported in a wide

variety of muscles, including the masseter muscle (for review: Calancie & Bawa, 1990). Thus, there seems to be a general agreement that motor unit recruitment during slow voluntary contractions is size dependent and orderly.

Although it is now generally accepted that motor units are recruited in an orderly fashion, which correlate with their size, controversy remains regarding the identity of the factors that determine a unit's functional threshold. A number of studies have questioned Henneman's view that a motoneuron size per se determines its susceptibility to discharge. Motor unit type, classified according to number of electrical and mechanical characteristics may be more appropriate as an indicator of functional threshold than motoneuron size or axon conduction velocity (Fleshman *et al.*, 1981; Kernell & Monster, 1981). Burke (1981) has taken the view that a number of factors combine to determine a motoneuron functional threshold, including the organisation of the synaptic input to the motoneurons, and the interactions of this input with the intrinsic motoneuron properties (absolute voltage threshold for action potential generation, absolute true resting membrane potential, membrane accommodation to depolarising currents and membrane processes controlling refractoriness).

A variety of inputs to motoneuron pools has been used to examine whether the distribution of an input goes along with the size principle. Many experiments are in agreement with the expectations of the size principle (Henneman, 1957; Henneman & Olson, 1965; Henneman *et al.*, 1965a,b; Clamann *et al.*, 1974a,b). However, in all of these experiments, the distribution of the synaptic inputs was determined by recording

the postsynaptic potentials. Since the input impedance of the cell can alter the size of the postsynaptic potential, it does not indicate the magnitude of the input current received by the cell. A more appropriate method may be to record the actual synaptic current received by the cell. For example, Heckman and Binder (1990) found that the Ia effective synaptic current was not uniformly distributed to motoneurons of the medial gastrocnemius muscle in cat. The high input impedance motoneurons (small motoneurons) tended to receive about twice as much Ia effective synaptic current as the low input impedance motoneurons (large motoneurons). The differentially distributed Ia input acts to strengthen the differences created by the intrinsic properties of motoneurons, hence, it enhances the size principle. This would maximise the usage of fatigue-resistant units, and this lower input-output gain would be advantageous in movements requiring a high degree of precision - a precisely controlled movement with low force level and slow speed.

Besides the group Ia muscle afferent input, evidence has shown that cutaneous afferent input is not distributed equally to motoneurons since it favours large motoneurons (Grimby & Hannerz, 1968,1976; Kanda *et al.*, 1977; Stephens *et al.*, 1978; Datta & Stephens, 1981; Garnett & Stephens, 1981). Reversal of recruitment order has been reported by Kanda and colleagues showing that in the decerebrate cat, stimulation of cutaneous afferents reversed the order of recruitment of motor units produced by muscle stretch (Kanda *et al.*, 1977; Kanda & Desmedt, 1983). In human first dorsal interosseous studies, cutaneous stimulation caused an increase in the recruitment threshold of units normally recruited at contraction strengths below 1.5 N and a decrease in the recruitment threshold of units recruited at contraction strength above



1.5 N (Garnett & Stephens, 1981). With the aid of cutaneous stimulation, therefore, the subject could recruit the unit which was previously recruited at a higher contraction level (Garnett & Stephens, 1981; Kanda & Desmedt, 1983). This behaviour is important in ballistic movements, or actions requiring rapid alternation of agonist and antagonists in animals (Smith *et al.*, 1980), or in skilled manipulatory activities of the human hand (Garnett & Stephens, 1981; Kanda & Desmedt, 1983).

Motoneuron innervation in the human jaw muscles originate from the trigeminal motoneuron pool. Excitatory inputs from cortical neurons converge there (Lund, 1991; Donga & Lund, 1991) and as the muscle contracts, corticobulbar cells progressively deliver more current until individual motoneurons reach their activation threshold and commence firing. Consistent patterns of motor unit recruitment have been reported in the jaw muscles of human and non-human primates. Units recruited first have lower twitch tensions and activation thresholds than those recruited later (Desmedt & Godaux, 1975; Yemm, 1977; Goldberg & Derfler, 1977; Clark *et al.*, 1978). The activation thresholds and the force outputs of human jaw muscle motor units seem to be correlated over this recruitment range (Yemm, 1977; Goldberg & Derfler, 1977; Nordstrom & Miles, 1990). However, their activation thresholds notoriously vary with task and time (Nordstrom & Miles, 1990). The recruitment order may change when powerful motor commands to the motoneuron pool activate many motor units almost simultaneously (Desmedt, 1983). Deordered recruitment can also occur during slow ramp contractions. This is not unique to the jaws and has been reported in multifunctional human limb muscles acting as prime mover or synergists (Desmedt, 1981; Schmidt & Thomas, 1981; Thomas *et al.*, 1987). In the human masseter and

temporalis muscles, it is common to find motor units contributing to more than one task. There can also be regional differences in this behaviour (McMillan & Hannam, 1992). Task specificity also manifests itself in jaw muscle motor unit recruitment thresholds. Hattori et al (1991) have shown that thresholds do differ with task and the direction of force application by using a transducer to record bite force in three dimensions. Masseter motor unit thresholds were very sensitive to bite force direction. Changes in motor unit recruitment order and threshold for activation suggest flexibly-organised synaptic inputs to the motoneuron pool. In limb muscles, cutaneous afferents affect different-sized motor units selectively, but it is not known how similar inputs affect human jaw motor unit recruitment.

There are several similarities between periodontal mechanoreceptors and cutaneous receptors:

- Morphology: periodontal mechanoreceptors are Ruffini-like receptors which also form one of the structures of cutaneous receptors.
- Synaptic connection: they both have polysynaptic connection to motoneurons.
- Function: they both receive the information about the objects being held or bitten on and modify the activities of motor units.

No studies dealing with the distribution of periodontal mechanoreceptor afferents to the motor units of jaw muscles has previously been performed. Therefore, the effect of periodontal stimulation on the recruitment order of jaw motor units remains unclear.

### **2.4.2 Firing Rate of Motor Units**

During voluntary muscle contraction, motor unit firing rate varies with the force produced by the whole muscle (De Luca *et al.*, 1982; Edström & Grimby, 1986). De Luca *et al.* (1985) suggested that increased excitatory inputs to the motoneuron pool increase the firing rate of active motor units collectively. The finding that the motoneuron pool is modulated as a whole has given rise to the expression, 'common drive'. Thus, smooth muscle contraction is a consequence of the relationship between motoneuron size which determines unit recruitment threshold, and common drive which initiates motor unit activity and determines firing rates. Almost all units may discharge tonically or phasically depending on the prevailing behavioural set (Nordstrom & Miles, 1991a,b). In both human and experimental animal studies,  $\alpha$ -motoneurons tend to fire relatively consistently at slow rates when activated tonically near the threshold (Burke, 1981). The lowest sustainable firing frequency appears to be around 6-8 Hz (Derfler & Goldberg, 1978; Johansson *et al.*, 1988), and this is roughly equivalent to the firing rate at which motor unit twitches begin to fuse (Kernell, 1974).

### **2.4.3 Interaction between Recruitment and Rate Modulation**

The relative contribution of motor unit recruitment and rate modulation in the control of forces varies between muscles. As a general rule, recruitment is completed in small muscles of the hand below 50% of maximal force (Milner-Brown *et al.*, 1973c; Kukulka & Clamann, 1981). Further increase in force by these muscles is accomplished by increasing the firing rate of the active units. Larger muscles rely on recruitment over a greater proportion of the force range, perhaps as high as 80%

(Kanosue *et al.*, 1979; Kukulka & Clamann, 1981; De Luca *et al.*, 1982). In large, powerful limb muscles, motor unit recruitment can continue up to 90% of maximum voluntary contraction (MVC) (De Luca, 1985). In the human masseter, motor units seem to rely more on rate coding to increase muscle contraction, with approximately 50% of the masseter motor unit are recruited at 10-20% of its MVC (Derfler & Goldberg, 1978; Clark *et al.*, 1978). This is similar to the small muscles of the hand, where most motor units are recruited below 50% of MVC and where rate modulation is used to further increase the force (De Luca, 1985).

The corollary of this is the observation that the dynamic range of steady firing rates are much greater in motor units of rate-coded muscles, such as first dorsal interosseous (9 Hz - 40 Hz, De Luca *et al.*, 1982), than in larger muscles relying primarily on recruitment such as soleus (6 Hz - 10 Hz, Mori, 1973). It has been suggested that rate-coding offers an advantage when fine control of force is required, such as in the finger muscles, because it allows smaller increments to be added to the total force.

The human masseter apparently is an exception to this general rule. Although it is a powerful muscle, it is heavily reliant on rate-coding with a mean motor unit firing rate vary from 6 Hz - 26 Hz (Derfler & Goldberg, 1978).

## 2.5 METHODOLOGICAL CONSIDERATIONS

### 2.5.1 *Subjects*

The most commonly used experimental subjects in reflex studies are the cat, on account of its convenient size and the large amount of basic data available on the organisation of its nervous system. Occasionally, experiments have been carried out on unanaesthetised animals, but more usually anaesthetised or decerebrate animals are used. Anaesthetics interfere with synaptic transmission and therefore inevitably affect reflex responses (Matthews, 1975).

Decerebration permits investigation of synaptic activity in the brain stem and spinal cord without anaesthetic. However, decerebration causes a considerable change in the excitability of cells within the brain stem and spinal cord, in particular, a facilitation of the stretch reflex in extensor muscles. These effects depend to some extent on the level and method of decerebration employed (Matthews, 1975).

The experimental procedures which can be used in humans are more limited than those used in animals, but it has been possible to devise experiments to look for mechanisms in humans corresponding to those found in animals. Human subjects can be asked to contract different muscle groups while the reflexes are observed superimposed upon the voluntary contractions. The manifestations of the reflexes often depend on the level of activity in the muscles; a low level of background activity may be used to reveal excitatory effects which are not apparent, and inhibitory effects which cannot be observed if there were no background activity present. All subjects used in current studies had normal dentition with no history of oro-facial

neuromuscular dysfunction or orthodontic treatment. The experiments were approved by the Human Ethics Committee of The University of Adelaide.

### **2.5.2 Stimulation Methods**

Stimulating one afferent type without affecting others has been a great challenge for a number of years (Türker *et al.*, 1999).

#### ***Electrical stimulation***

Since the peripheral processes of the periodontal mechanoreceptors belong to the alveolar nerves, many investigators have attempted to study the reflex effects by electrically stimulating these nerves. One complication of this approach is that the nerves contain a variety of different afferent types with overlapping fibre diameters and conduction velocities overlap (Dessem & Taylor, 1989). In an effort to maximise the specificity of the stimulus to the periodontal mechanoreceptors, some researchers have dissected the branches of the inferior alveolar nerve which are directed towards the teeth. Low-intensity stimulation of these branches provide a more selective stimulation of the periodontal mechanoreceptors than stimulation of the whole inferior alveolar nerve. However, some of the large-diameter nerve fibres innervating the gingiva, periosteum and tooth pulp are still activated (Dessem & Taylor, 1989).

Direct electrical stimulation of the periodontal ligament has also been used to activate periodontal mechanoreceptors (Anderson & Mahan, 1971; Bailey & Capra, 1988; Shigenaga *et al.*, 1988b). Since afferent modalities other than the mechanoreceptors are contained in the periodontal ligament (Sakada & Kamio, 1971; Mei *et al.*, 1977),

stimulation of the periodontal ligament without reference to the stimulation strength is unlikely to be limited to the periodontal mechanoreceptors. An additional difficulty with this method, is to prevent the spread of stimulus current to the pulpal fibres at the apex of the tooth and afferents in the surrounding gingiva (Dessem & Taylor, 1989).

Therefore, it has not been possible to use electrical stimuli to excite just periodontal mechanoreceptor fibres without the risk of simultaneously exciting other types of fibres.

### ***Mechanical stimulation***

The most functional way of activating the mechanoreceptors surrounding the teeth could be done by mechanical displacement since this is how they are activated during mastication and biting.

A tap to a tooth often brings changes in the EMG of the jaw muscles and hence has been used for many years to study the connection of the periodontal afferents to the motoneurons of jaw muscles (Matthews, 1975). However, it has been suggested by many researchers that this type of stimulus also activates several receptor systems under study (Hannam & Matthews, 1969; Cash & Linden, 1982b). For example, a tap can stimulate the receptors in the area of application, such as the cutaneous receptors (Bailey *et al.*, 1979), auditory receptors (van Steenberghe & Van Der Glas, 1981), distant palatal receptors and periodontal mechanoreceptors (Beaudreau *et al.*, 1969). The tap can also stimulate vibration, stretch and position sensitive receptors in and around the jaw region (Lund *et al.*, 1983), which can induce excitation of jaw closing

muscles and transitory synchronisation of jaw closing muscle motor units. As evidence that a vibration could produce these changes in jaw closing muscle activity, it has been shown that tapping heavily on a small metal disc pressed against the skin of the forehead produces very similar effects to those caused by tapping a tooth (Hannam *et al.*, 1970). Finally, a tap can stimulate the vibration-sensitive receptors in the inner ear (Meier-Ewert *et al.*, 1974; Sato *et al.*, 1994), and was confirmed when local anaesthetic was infiltrated around the tooth (van Steenberghe & Van Der Glas, 1981). The inhibitory reflex reduced in size but did not disappear (Matthews, 1975). Playing white noise (80 dB - 90 dB) in to the ears of these subjects, completely abolished the reflex.

Even when the applied force has been controlled to stimulate the periodontal mechanoreceptors, it is difficult to understand why several researchers found different reflex connections between the periodontal mechanoreceptors and the jaw muscles (Brodin *et al.*, 1993b; Türker *et al.*, 1994; Louca *et al.*, 1996b). From microneurography studies, some receptors in the trigeminal system respond to slowly-rising forces while others are activated by a rapidly-rising component of the force stimulus (Trulsson & Johansson, 1994). The stimulus profile, therefore, may be very important in determining the response. If the stimulus profile contains only a slowly-rising component, then it is likely that it will activate the slow rate sensitive receptors. When the stimulus profiles has more fast components, the receptors that are activated will also include the fast rate sensitive receptors of the system. However, mechanical stimulation is not very easy to standardise since its application also requires fine control of the duration, intensity and exact location of the stimulus. Furthermore, the



profile of the stimulus shape, including the rate of rise of the stimulus, is difficult to standardise because of changes with the physical relationship between the stimulating probe and the stimulated area. Therefore, there is a need to standardise the mechanical stimulus, to facilitate the study of the reflex connection between the periodontal mechanoreceptors and the jaw muscle activity.

### ***2.5.3 Recording and Analysing Reflex Response***

#### ***Surface electromyography***

The simplest way of obtaining evidence of muscle activity in humans is to attach surface electrodes to the skin and record the small electrical potentials set up by action potentials flowing in the underlying muscles. A record of this type is referred as surface electromyography (SEMG).

SEMG is easy to use and provides important results. However, a single trace of SEMG does not represent reflex connection of the stimulated afferent to the muscle. Several manipulations have been performed to convert SEMG records into quantifiable forms. The most common technique is full-wave rectification and averaging of the SEMG around the time of stimulation. This approach has the capacity to illustrate the strength of the reflex response (Goldberg, 1971). Different researchers used different methods to convert the rectified-averaged SEMG for this purpose. For example, Goldberg (1971) used a planimeter to measure the area of the rectified averaged SEMG record; van der Glas et al. (1984) used the sum of the absolute surfaces outside the confidence interval (total normalised surface, TNS); Evans et al. (1989) used the percentage

modulation of the mean SEMG level as the calibration bar; Cadden and Newton (1994) calculated the area to indicate the percentage change.

Although the method of SEMG recording has the advantage that it is simple, it has some limitations. It cannot be used to record the activity from muscles which are not immediately beneath the skin; the further the muscle fibres are from the electrodes, the smaller the recorded potentials tend to be. It is tempting to assume that the amplitude of a SEMG can be used as a measure of the amount of muscle activity present. Since the potential recorded from any one active muscle fibre depends on its distance from the recording electrodes, an increase in the amplitude of a SEMG does not necessarily indicate a change in the number of active fibres, or their frequency of firing, but may be due to a change in the position of the active fibres (Matthews, 1975).

Quantifying the reflex from the SEMG data can be difficult for several reasons. The synchronisation type error occurs due to phase advancing or delaying the occurrence of single motor unit action potentials in bulk. For example, an excitatory postsynaptic potential (EPSP) can cause a phase advancement of the occurrence of the action potentials. The phase advanced events induce a peak in the SEMG record at the reflex latency and this peak can then induce several peaks separated from each other by about one interspike interval as the action potentials fire again (Türker & Cheng, 1994). Similarly, delayed single motor unit action potentials by an inhibitory postsynaptic potential (IPSP), induces a peak in the SEMG. Therefore, an increase in the SEMG level, following an inhibitory reflex, may not always indicate an excitatory synaptic connection, but may be caused by the synchronous delayed re-occurrence of the single

motor units (Miles & Türker, 1987; Bonte & van Steenberghe, 1989). The count related error can also affect the interpretation of the reflex event. The decreased activity in the SEMG immediately after an excitatory reflex may be due to the phase advanced spikes rather than an inhibitory reflex (Türker *et al.*, 1997). During this time of relative 'silence', many of the single motor unit action potentials cannot fire. Calculation of the CUSUM from the rectified averaged SEMG partly solves the problem of authenticating the secondary response (Brodin *et al.*, 1993b). For example, the phase delayed spikes occur again which brings the CUSUM trace back to the zero line. Any further increase in the CUSUM recorded above the prestimulus fluctuation and within the reaction time to the stimulus, reflects genuine extra activity. This can only come from an excitatory synaptic connection between the stimulated afferent and the motoneurons that innervate the muscle.

Furthermore, SEMG gives no information about the behaviour of individual motor units. High threshold and low threshold motor units may respond differently to a stimulus (Garnett & Stephens, 1980), but this cannot be seen on the SEMG. These limitations of SEMG can be overcome by recording from single motor units (Miles, 1997).

### ***Bite force recordings***

The most reliable measure of the output of the masticatory system is, of course, the force exerted between the teeth. The reflex response of bite force will not be affected by the placement of electrodes nor is the bite force affected by the artefacts of SEMG, and represents the net response of the masticatory system to a mechanical tooth

stimulation. Reflex response of bite force demonstrates more clearly whether periodontal mechanoreceptors provide a positive feedback to jaw muscles.

### ***Single motor unit recordings***

The introduction of the single motor unit (SMU) recording technique into the experimental investigation of jaw reflexes in humans has greatly increased the understanding of the neural mechanisms and circuitry involved. Although the technique is demanding, it offers several advantages over more conventional surface recordings. Recording SMU action potentials from muscles has the distinct advantage that it allows observation of the output of individual motoneuron since there is a one-to-one relationship between these two events. Furthermore, SMU recording is artefact free since the action potentials are all-or-nothing events.

Although recording SMU activity from human muscles is easy, quantifying the reflex response has been a challenge with numerous techniques developed (Garnett & Stephens, 1980; Awiszus *et al.*, 1991; Türker *et al.*, 1994). In order to quantify reflex responses, the firing probability of the SMU following a stimulus has been used by investigators (Garnett & Stephens, 1980; Kudina, 1980). A peristimulus time histogram (PSTH) is constructed from the firing of one motor unit over many trials to assess the effect of the stimulus on the firing probability. In general, the counts in the PSTH have been used in a large number of calculation algorithms in an attempt to determine the value of the reflex response. In the PSTH approach an excitation of an motor unit as a result of a stimulation elicits a peak, whereas an inhibition induces a trough.

#### ***2.5.4 Recording the Size of Single Motor Unit***

To determine whether the recruitment of motoneurons occurs according to the size principle, it is necessary to be able to measure motoneuron size. Estimation of motoneuron size has to be indirect in humans. The number and size of the muscle fibres of a motor unit (muscle unit) is an indicator of the size of the motoneuron innervating these muscle fibres (Buchthal & Schmalbruch, 1980; Henneman, 1981). 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. The strengths and weaknesses of the methods for determining muscle unit size or motoneuron size are discussed below.

##### ***Spike triggered averaging of force***

Large motoneurons innervate muscle units that have a large number of fibres. These larger muscle units develop higher mechanical forces. Thus the relative size of the muscle units can be determined from the force they develop. To determine the contribution of a muscle unit to the force output of a muscle, spike triggered averaging (STA) has been used frequently (Stein *et al.*, 1972). In STA, the force record from a muscle is averaged, using the firing of a motor unit as the trigger. It is assumed that the averaged force is an accurate measure of the tension that is developed by the muscle unit because other units fire asynchronously in relation to the unit of interest, especially at low force levels. There are limitations to the STA technique (Calancie & Bawa, 1990), particularly in the jaw muscles, as it 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 and not accurate,

due to the complex internal architecture of the masseter (Hannam & McMillan, 1994). There was an unexpected increase in the twitch amplitude of masseter motor units when the firing rate decreased below approximately 10Hz in one study (Nordstrom *et al.*, 1989). STA recordings are contaminated by the presence of co-contraction of the jaw openers and closers (McMillan *et al.*, 1990), that occurs during most functional activities (Van Eijden *et al.*, 1990). Goldberg and Derfler (1977) described the practical difficulties of extracting twitch characteristics from STA of force since the signal-to-noise ratio became very high with units producing low force levels, and the high firing frequency of these units even close to their threshold results in fusion of the twitches and difficulty determining peak tension.

### ***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 the masseter (Hannam & 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 the 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 & Godaux, 1977) and fatigue (Nordstrom & Miles, 1991b) also affect recruitment thresholds in the jaw muscles. As described by van Eijden *et al.*, the contribution of the masseter to jaw closing force 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 (Van Eijden & Raadsheer, 1992).

### ***Spike amplitude of single motor unit action potential***

Goldberg and Derfler (1977) studied the relationship between spike amplitude, twitch tension and recruitment order in the masseter. They found a positive relationship between spike amplitude and recruitment order. The 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, using spike amplitude as an indicator of muscle fibre size, its limitation must be taken into account, as the amplitude of a motor unit action potential is highly dependant on the distance between the recording electrode and the fibres of the motor unit (Miles *et al.*, 1986).

### ***Representation of Macro EMG (MacroRep)***

An intramuscular recording technique for the study of motor units of different sizes has been described using a modified single fibre EMG electrode, Macro EMG electrode (Stålberg, 1980). The electrical activity of the shaft of the electrode against a far-away non-muscular site, 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 to the Macro EMG (MacroRep, Scutter & Türker, 1998) indicates the cross-sectional area of the muscle units. An intramuscular electrode is preferable when using this procedure; although the MacroRep can be produced by averaging the surface EMG with the firing of a motor unit, the MacroRep amplitudes 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, 1993).

The amplitude and area of the MacroRep are both positively correlated to the size and number of muscle fibres in the muscle unit. The area is less affected by electrode position whereas the amplitude is more precise in cases of poor baseline measurements. Overall, the peak-to-peak amplitude is the most important parameter of the MacroRep (Stålberg, 1983). As the number and size of the muscle fibres of a motor unit are good indicators of the size of the motoneuron innervating the muscle fibres (Buchthal & Schmalbruch, 1980; Henneman, 1981), the MacroRep can be used as a reliable indicator of muscle unit and motoneuron size.

MacroRep has been used in recruitment studies by several 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 & Spellman, 1996).



# **CHAPTER 3**

## ***STIMULUS PARAMETERS FOR ELICITING EXCITATORY MASSETERIC REFLEXES IN HUMANS***

### **3.1 INTRODUCTION**

It has been generally accepted that the main response of a fast mechanical stimulus ('tap') to a tooth is reflex inhibition, which may be followed by reflex excitation of jaw closing muscles both in humans and animals (Van Der Glas *et al.*, 1985; Dessem *et al.*, 1988; Bonte & van Steenberghe, 1989; Bjørnland *et al.*, 1991; Bonte *et al.*, 1993; Sato *et al.*, 1994). Recently it has been shown that, in contrast to a tap applied at a rate of about 1300 N/s, a push stimulus applied at about 50 N/s can reflexly facilitate the activity of the human masseter (Brodin *et al.*, 1993b; Türker *et al.*, 1994).

However, other researchers using similar push stimuli could not induce this sole excitatory reflex (Bonte, 1993; Louca *et al.*, 1996a). Instead, they always induced an inhibitory reflex which in some cases was followed by an excitatory reflex. Even in the previous experiments of Brodin *et al.* (1993b) and Türker *et al.* (1994), not every push trial induced a sole excitatory reflex. In many trials, the excitatory reflex response was preceded by an inhibitory reflex response. The likelihood of eliciting a sole excitatory reflex was about 40 % in both surface and single motor unit experiments (Brodin *et al.*, 1993b; Türker *et al.*, 1994).

Since a sole excitatory reflex could not be induced by other researchers and only could be induced in about 40 % of previous push experiments, it led to the question as to whether some other factors affect the type of reflex response of jaw closing muscles to stimulation of the periodontal mechanoreceptor. The push stimulation technique needs to be further investigated to determine the optimal parameters for eliciting sole excitatory reflex response of the masseter and this was the aim of this investigation. A preliminary account of this work has appeared as an abstract (Yang & Türker, 1995).

## **3.2 METHODS**

These experiments were approved by the Human Ethics Committee of The University of Adelaide. The subjects were 7 adults (4 males and 3 females) aged 19-36 years, most of whom were subjects on repeated occasions. All subjects had normal dentition with no history of oro-facial neuromuscular dysfunction or orthodontic treatment.

### ***3.2.1 Experimental Apparatus***

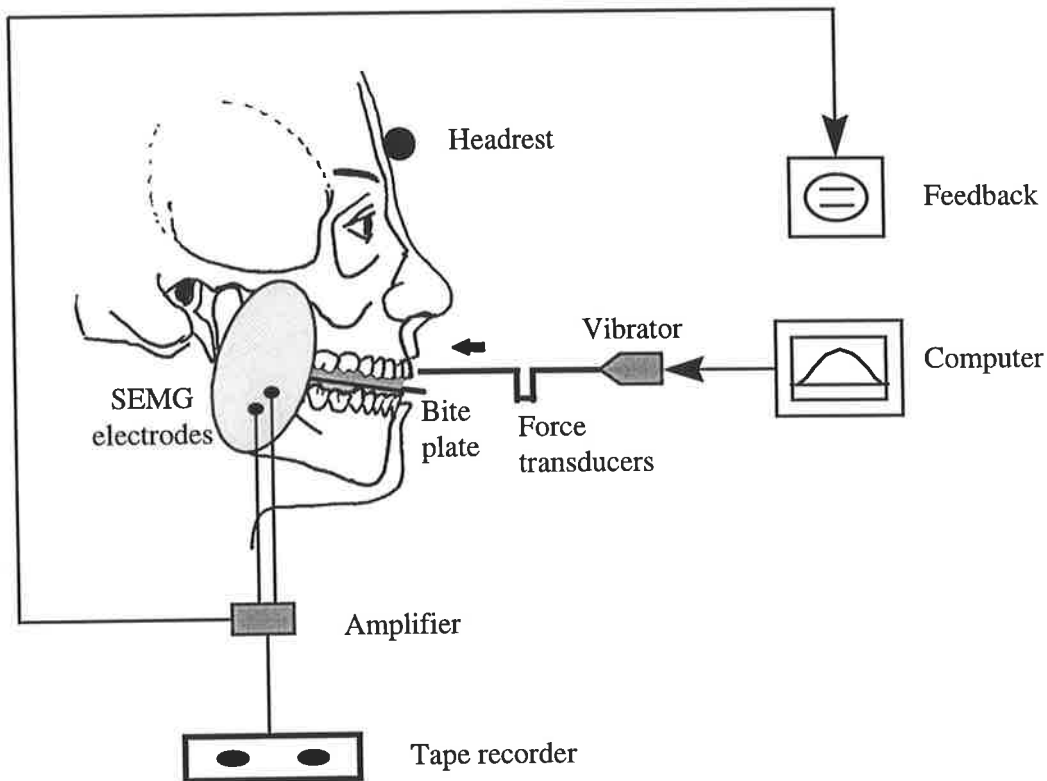
The subject was seated comfortably with his/her upper teeth held in fixed relation to a Perspex<sup>®</sup> probe mounted on the moving coil of an electromechanical vibrator. This fixed relation was achieved by asking the subject to bite into an impression of his/her upper and lower teeth, which was attached to a rigid frame (Figure 3.1). The impression material which is around the upper left lateral incisor was cut away to allow that tooth to be stimulated by the probe. The position of the subject's head was

further secured by asking the subject to rest his/her forehead on a horizontal bar (headrest).

### ***Stimulation***

The stimuli were pushes, applied orthogonal to the labial surface of the left lateral incisor tooth. The force applied to the tooth was measured with a strain gauge mounted in series with the stimulating probe. The peak force for pushes was about 2.5 N (range 2.2 - 2.7 N). The shape, amplitude and frequency of the stimulus wave was produced using a wave-generating computer program. To avoid adaptation and prediction, the computer program was set to initiate random stimuli with the inter-stimulus interval varying between 2 and 5 seconds. Several waveforms of stimulus were used to drive the stimulating probe as follows:

- (1) trapezoidal wave, with a 50 ms rise-time and a total duration of 150 ms (Type-T);
- (2) half sinusoidal wave lasting for 150 ms (Type-S);
- (3) half sinusoidal wave with slower rise and slower fall lasting for 200 ms (Type-SS);
- (4) Type-SS wave with a rubber attachment over the tip of the probe (Type-SSR).



**Figure 3.1 Diagrammatic illustration of experimental apparatus**

The subject bit into an impression of his/her teeth on a bite plate which was mounted onto a rigid frame. The position of the subject's head was further secured by asking the subject to rest his/her forehead on a headrest. The subject controlled the level of muscle activity with the help of SEMG feedback from the ipsilateral masseter. The shape of the stimulus profile was determined on a computer, which sent this wave to a vibrator to stimulate the tooth. The strength and the profile of the stimulus were measured by a force transducer placed in series with the vibrator. The SEMGs of ipsilateral and contralateral masseter were recorded simultaneously into a tape recorder for off-line analysis.

### ***Recording***

Bipolar electrodes were placed to record the surface electromyogram (SEMG) from the ipsi- and contralateral masseter muscles in the bandwidth 20-1000 Hz. The ipsilateral SEMG signal was rectified and low-pass filtered at 0.1 Hz and was displayed on an oscilloscope screen for feedback to the subject. The subject was asked to bite in such a way as to keep the level of activity of the ipsilateral masseter muscle at a pre-determined level, which was 10 % (range 5 -15%) of the maximal voluntary contraction (MVC).

#### ***3.2.2 Protocols***

At the start of each experiment, the subject was asked to bite into the impression of the teeth fixed on a rigid frame. The full-wave rectified SEMG was measured on an oscilloscope during a maximal voluntary contraction effort. This was regarded as a reference level of 100% MVC. During all stimulation sequences, the subject contracted the masseter muscles to produce 10% MVC level.

#### ***No Preload vs Preload***

In **No preload** trials, the probe was just touching the tooth and the preload was very close to zero. This gentle touch of the probe to the tooth was found necessary to avoid very large fast-force components from developing on the tooth. Such large fast-forces were observed when the probe hit the tooth from a distance. It was noted that even a push stimulus that started from a few millimetres from the tooth induced a very rapidly-rising force as the probe accelerated towards the tooth. Such rapidly-rising

force resembled the tap stimulus. In **Preload** trials, the probe was held continuously in contact with the tooth by a prestimulus force of about 0.5 N (range 0.2 - 0.6 N).

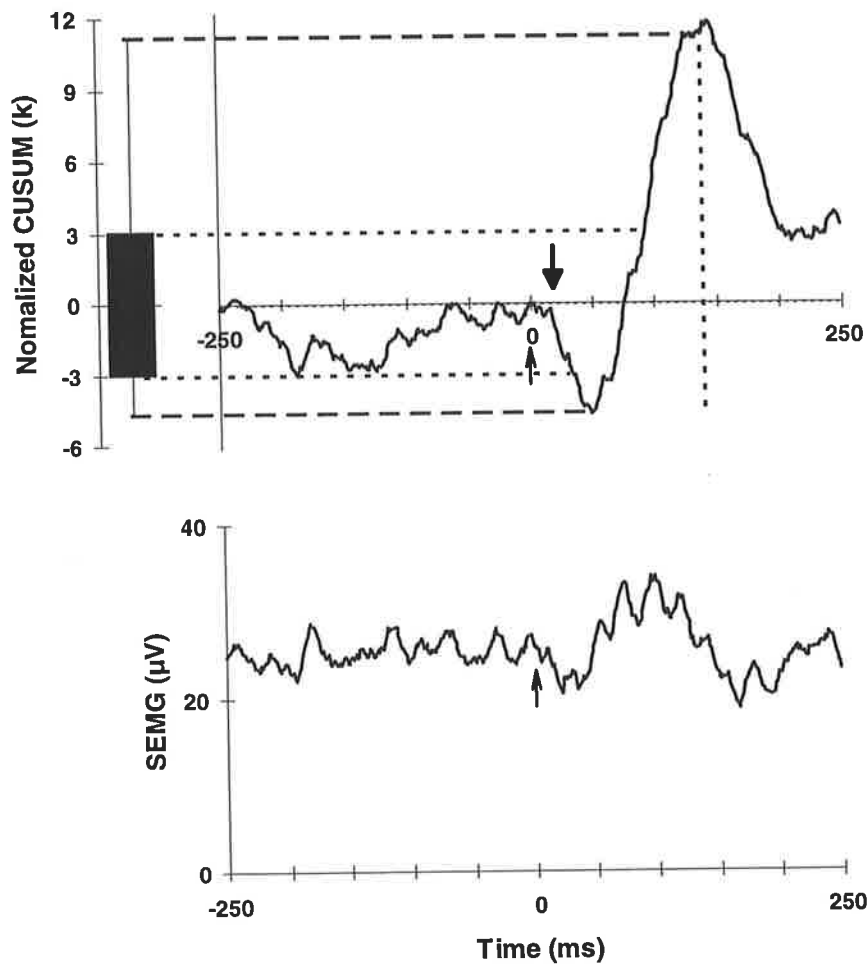
### *Different stimulus profiles*

Four different stimulus profiles (Type-T, -S, -SS, -SSR) were tested. Subject was asked to maintain 10% MVC level, while 0.5 N preload was given before the actual stimulation force was applied.

When the position was stable, 50 stimuli of mechanical push force were applied to the upper left lateral incisor. Three successive experimental trials of 50 stimuli were performed. Between the trials, the subject rested for 30 seconds on the frame. After three consecutive trials the subject came off the frame and rested for five minutes before the next 3 successive trials. In each experiment, 18 trials were usually performed. In all experiments the sound of the mechanical stimulus was masked by white noise played into earphones at 80 - 90 dB. The SEMG and the push force data were recorded on a digital tape recorder for off-line analysis.

### *3.2.3 Surface EMG Analysis*

The SEMG recording of ipsilateral and contralateral masseter was full-wave rectified, filtered (DC - 400 Hz) and sampled at 1 kHz before averaging (12 bits resolution), and CUSUM of the averaged SEMG record was constructed (Figure 3.2).



**Figure 3.2 SEMG and its CUSUM**

The rectified + averaged ( $n=50$ ) SEMG of ipsilateral masseter muscle (bottom) and its CUSUM (top) are illustrated. The small arrows indicate the timing of the stimulus (time zero). From the prestimulus period of the CUSUM records, maximal positive and negative deflections were obtained. The larger of the two values was used to make a symmetrical box (■) for the CUSUM record. The box therefore illustrated the largest deflection to either side of the zero line during the prestimulus period ('error box'). Any poststimulus deflection that was larger than the error box and that occurred within 140 ms (vertical line indicating reaction time) after the stimulus, was considered a reflex response. To determine the reflex latency, CUSUM record going below the dotted line was extrapolated back to the baseline and the timing of the first deflection in the same direction was noted. For example, in this figure, there was an inhibitory reflex response at a latency of about 20 ms indicated by large downward arrow. Calibration of CUSUM is in the multiples of prestimulus average bin value (k).

The following procedure was performed to obtain normalised CUSUM: SEMG records for 50 identical stimuli were averaged ( $\pm 250$  ms around the time of stimulation; bin width = 250  $\mu$ s); the average bin value (**k**) for the prestimulus period (between -250 and 0 ms) was calculated; for the poststimulus period (0 - 250 ms), the value in each bin (**a**) was then subtracted and divided by the (**k**) value,  $[(a-k) / k]$  (Dividing the bin value by **k** normalised the CUSUM calculations and allowed the amplitude of the reflex response to be comparable between trials); the resultant values in each bin were then added to obtain normalised CUSUM of the averaged EMG trace,  $\Sigma[(a-k) / k]$ .

From the prestimulus period of CUSUM records, maximal positive and negative deflections were obtained. The larger of the two values was then used to make a symmetrical 'error box' (Figure 3.2). From the CUSUM records, the existence of a reflex response was determined by comparing deflections in the poststimulus CUSUM with the size of the error box. For the response to be considered a reflex, it needed to be larger than the error box and have occurred within 140 ms of the stimulus (the reaction time to this stimulus, Brodin *et al.*, 1993a). For example, in Figure 3.2, the CUSUM record goes below and then above the dotted line (representing the size of the error box) within 140 ms (vertical line at 140 ms).

### 3.2.4 Statistical Analyses

The incidences of eliciting a sole excitatory reflex (SER), a sole inhibitory reflex (SIR) and an inhibitory reflex that were followed by an excitatory reflex (IER) were compared when different stimulation patterns were used. A Chi-squared test was used



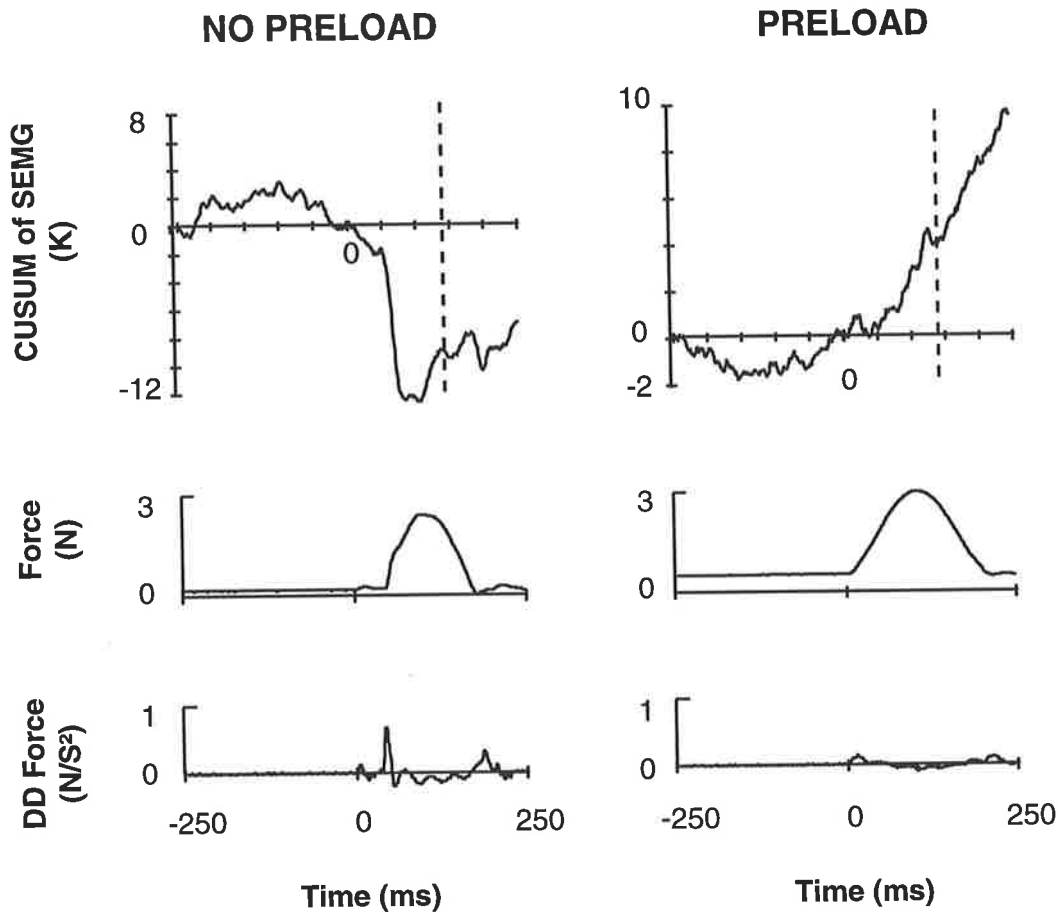
for comparing the reflex response of Preload and No Preload cases. A Polychotomous Regression test (McCullagh, 1980) was used for comparing ipsi- and contralateral masseter muscle responses, the stimulus wave forms and the effect of the change in force rate. The differences were accepted to be significant if the  $P$  value was less than 0.05.

### 3.3 RESULTS

Seven subjects took part in this study during 13 experimental sessions. Using the Polychotomous Regression test, it was established that there was no significant difference in the likelihood of eliciting any of the reflexes between the ipsi- and contralateral sides. Therefore, the probabilities of eliciting SER, SIR and IER were shown for both masseters in Table 3.1 and Table 3.2. When no reflex responses were observed, ie., the poststimulus CUSUM deflections within 140 ms time period were smaller than the error box, these instances were indicated as 'no reflex'.

#### 3.3.1 *Preload vs No Preload*

Using the same stimulation pattern, the likelihood of eliciting SER was significantly higher with preload than without preload (58 % versus 21 %;  $P < 0.001$ , Figure 3.3).



**Figure 3.3 The effect of preload on the reflex responses of ipsilateral masseter**

The CUSUMs of SEMG, stimulation force and double differentiated force profiles are illustrated. **No Preload** trials are shown on the left and **Preload** trials (0.5 N) are on the right. Note that in **No Preload** trials, the preload is not exactly zero as the probe gently touched the tooth preceding the push to prevent a tap-like effect. The effective force is illustrated in the middle while the double differentiated profile of that force (bottom) indicates the change in the force rate. The force calibration bars indicate 3 N for the effective force and 1 N/S<sup>2</sup> for change in force rate. The stimulus was applied at time zero. The vertical dashed line indicates the reaction time for this experiment. The number of stimuli averaged was 50 and the stimulus parameters were Type-SSR, 0.5 N preload, 2.5 N push force and 10 % MVC. Note that there is a significant decrease in SEMG in **No Preload** trial, but an increase in **Preload** trial.

The CUSUM records, push force profiles and the change in the force rate with and without the preload are illustrated in Figure 3.3. The force profile was differentiated twice (using a 7-bin moving window) to illustrate its fast and slow components. During the experiments, this was achieved by high-pass filtering (at 50 Hz) the force profile. Continuous monitoring of the filtered force profile on-line was necessary to detect any slippage of the probe due to a very small and otherwise undetectable change in the subject's head position relative to the probe. A slippage of the probe on the tooth surface, as with **No Preload** experiments, induced fast force components which were significantly associated with inducing SIR or IER (Figure 3.3).

Likewise, as shown in Table 3.1, the likelihood of eliciting sole inhibitory reflex (SIR) was lower with preload than without preload and this difference was significant (15 % versus 52 %;  $P < 0.001$ ).

**Table 3.1 Reflex responses as force applied with No Preload and with Preload**

	No preload	Preload
SER	21%	58%
SIR	52%	15%
IER	25%	19%
No reflex	2%	9%
Number of trials	52	156

In all cases the stimulus wave was Type-SS, or Type-SSR. Push force was 2.5 N, the background masseter muscle activity maintained at about 10% MVC. SER= sole excitatory reflex; SIR= sole inhibitory reflex; IER= inhibitory followed by excitatory reflex. Fifty stimuli were applied in one experimental trial.

### 3.3.2 Reflex Responses of the Masseter to Different Stimulus Profiles

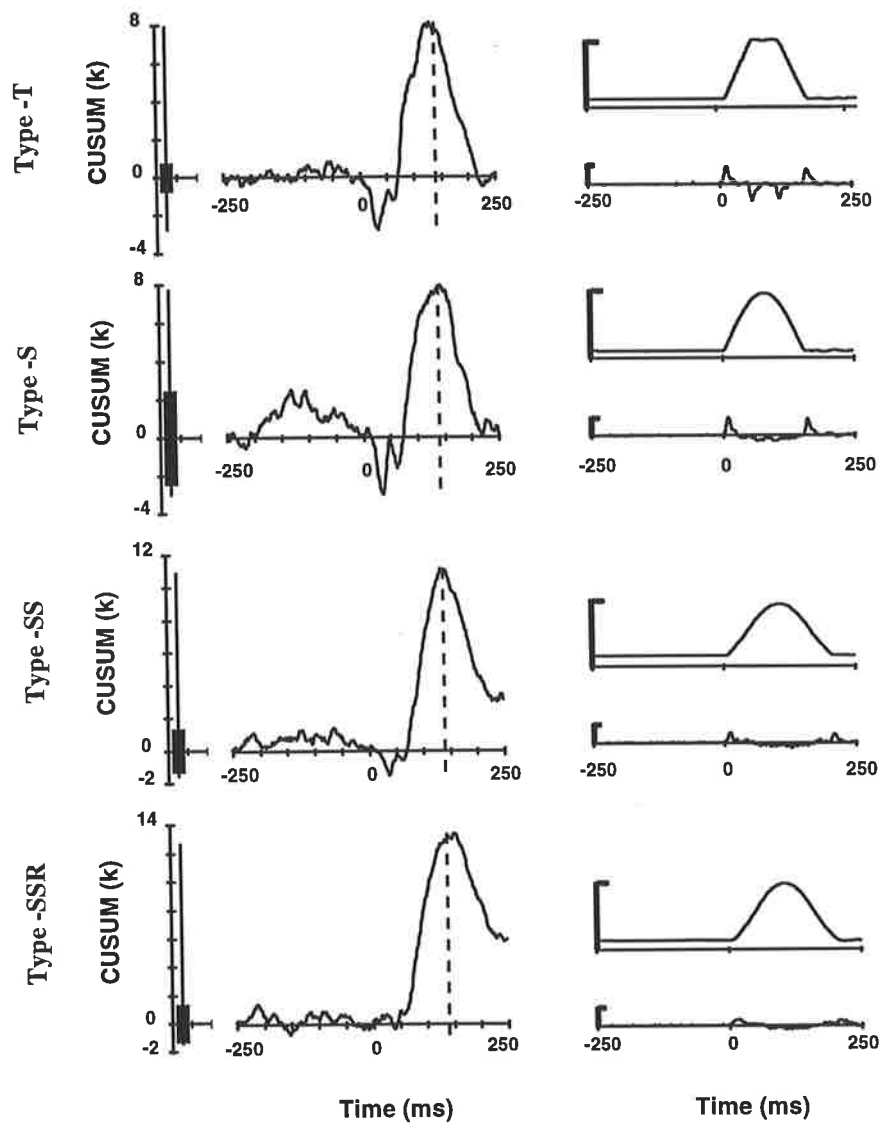
To compare the importance of the shape of the stimulus waves (Table 3.2 and Figure 3.4), the same standard protocol was used. This standard protocol required a preload of about 0.5 N and a push force of about 2.5 N. Also, the background level of activity in the masseter muscle was about 10 % MVC, and the stimulus probe was directed to the incisal edge of the tooth in order to mainly stimulate the apical periodontal mechanoreceptors (Linden, 1990).

**Table 3.2 Reflex responses to different stimulus profiles**

	SER (%)	SIR (%)	IER (%)	No reflex (%)	Number of trials
Type-T	37	30	28	5	60
Type-S	37	23	34	6	62
Type-SS	45	17	10	28	60
Type-SSR	50	28	2	20	70

The standard protocol (0.5 N preload, 2.5 N push force, 10% MVC) was used in all of the above experiments. SER= sole excitatory reflex; SIR= sole inhibitory reflex; IER= inhibitory followed by excitatory reflex. Fifty stimuli were applied in one experimental trial.

Figure 3.4 illustrates the effect of stimulation profile on the reflex responses. The force profiles that indicate the actual force that was delivered to the tooth are shown together with its twice differentiated records, which highlight the fast and slow components of the push force.



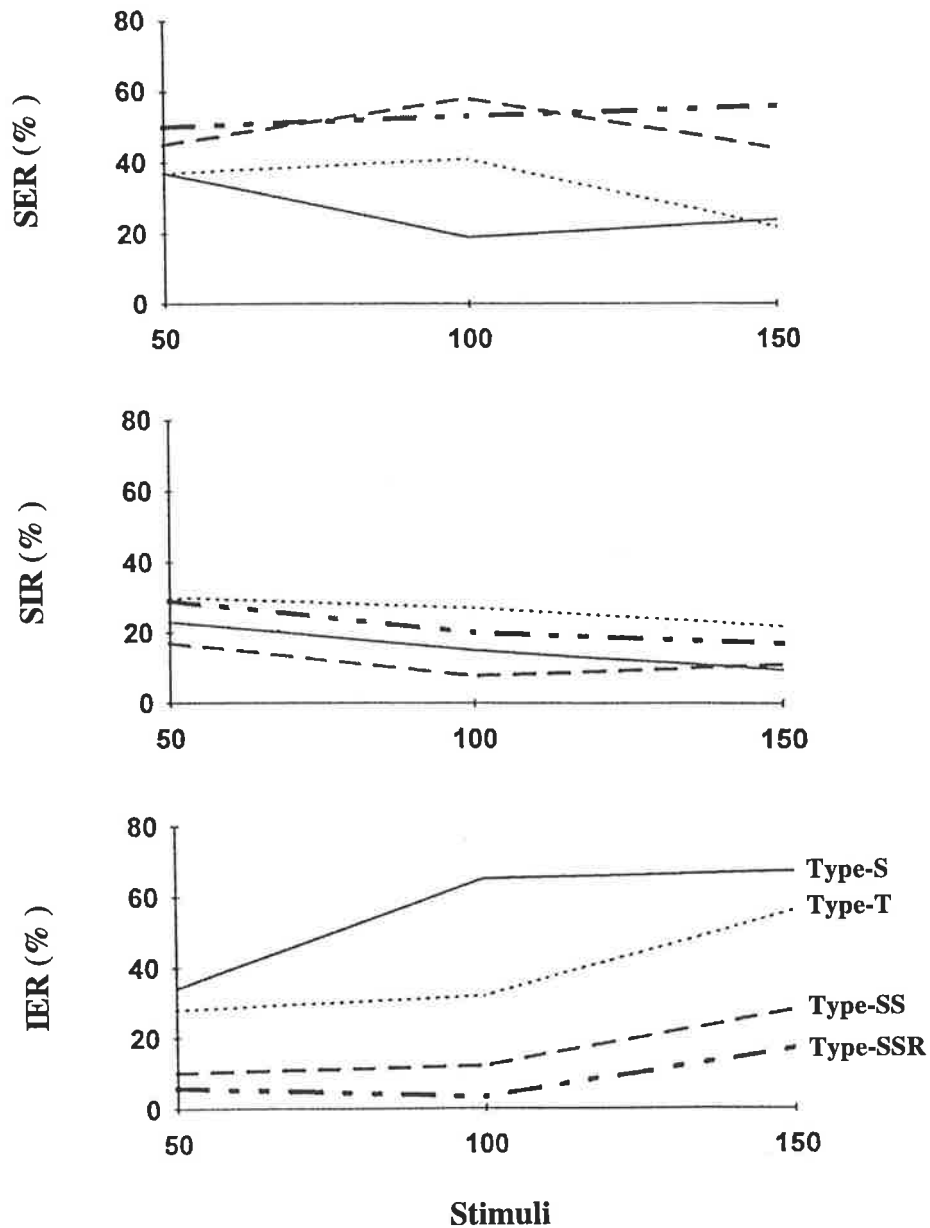
**Figure 3.4** Different stimulus profiles and the reflex response of ipsilateral masseter

The left column illustrates the CUSUMs of the ipsilateral masseter muscle. The right column indicates the profiles of stimulating forces. Stimulus was given at time zero. From the top to bottom: the CUSUM and the force profiles to **Type-T** stimulus wave; the CUSUM and the forces related to the **Type-S**; the CUSUM and force profiles related to **Type-SS** and the CUSUM and force profiles to **Type-SSR**. In all cases the number of stimuli used was 150 (ie. three identical trials were added in each case); the preload was 0.5 N; the push force was 2.5 N; all had incisal edge stimulation; all had contraction levels of about 10 % MVC. In each stimulus type, the top right trace is the force profile that is applied to the tooth and the lower part is the twice differentiated profile of that force indicating the change in the force rate. The force calibration bars indicate 3 N for the upper force record and 0.4 N/s<sup>2</sup> for the lower force record. The horizontal line under the force record indicates the 0 N force level.

Stimulation Types -SS and -SSR induced SER significantly more frequently and IER significantly less frequently compared with the Types -T and -S ( $P < 0.05$  in all cases). When the change in force rate was used to identify the stimulus shape, it was again found that as the change in force rate became faster, it was significantly less likely to induce SER and significantly more likely to induce SIR and IER ( $P < 0.05$  in all cases). The average change in force rate was  $0.35 \text{ N/s}^2$  for both Types -T and -S,  $0.2 \text{ N/s}^2$  for Type-SS and  $0.1 \text{ N/s}^2$  for Type-SSR. Therefore, Types -T and -S were more likely to induce SIR and IER, and less likely to induce SER.

### ***3.3.3 The Effect of a Number of Stimuli on the Incidence of Various Reflexes***

Figure 3.5 shows the effect of a number of stimuli on the percentage incidence of various reflexes. The results of the trials where 50 stimuli were used have been shown (Table 3.2). When two such trials with identical parameters (ie., same stimulus, same person and same day) were averaged, the points that lie above 100 stimuli were obtained. When results of three identical trials were averaged, the points that lie above 150 stimuli were obtained (Figure 3.5). With an increased number of stimuli, the percentage probability of detecting SER using stimulus Type-SSR increased. The percentage of SIR decreased in all stimulus types. The percentage of IER increased significantly when types -S and -T were used.



**Figure 3.5** The effect of the number of stimuli on the percentage success of various reflexes

The points that lie above 50 stimuli belong to the findings of individual trials that each used 50 stimuli (Table 3.2). When two or three trials with identical stimulus parameters were added, the points that lie above 100 and 150 stimuli were obtained. The finding of **Type-S**, **Type-T**, **Type-SS**, **Type-SSR** were indicated by different types of lines. Note that to obtain the probabilities of the various types of stimuli, the results of ipsi- and contralateral masseter were added in this figure. For 50 stimuli:  $n = 60, 62, 60$  and  $70$ ; for 100 stimuli:  $n = 22, 26, 26$  and  $30$ ; and for 150 stimuli:  $n = 18, 20, 18$  and  $18$  for Types -T, -S, -SS and -SSR respectively.

The same experimental protocol was repeated many times in order to assess the time-dependent changes in the reflex responses (habituation) and also to bring out subtle reflex effects by averaging. Habituation was not observed even when the same stimulus profile was delivered in ten consecutive trials. When trials with the same stimulus parameters were added, however, the size of the error box was reduced by the increase of the number of stimuli.

### 3.4 DISCUSSION

The reflex response to fast mechanical stimulus (tap) applied to a tooth has been studied by a number of researchers (Van Der Glas *et al.*, 1985; Dessem *et al.*, 1988; Bonte & van Steenberghe, 1989; Bjørnland *et al.*, 1991; Bonte *et al.*, 1993; Sato *et al.*, 1994). It has been argued that as well as stimulating the receptors in the periodontal space, the vibrations initiated by the stimulus can spread to vibration-sensitive remote receptors such as the muscle spindles (Hannam *et al.*, 1970; Orchardson & Sime, 1981) and the receptors in the middle ear (van Steenberghe & Van Der Glas, 1981; Van Der Glas *et al.*, 1988). However, a slowly-rising mechanical stimulus (push) is more likely to stimulate only the periodontal mechanoreceptors (Louca *et al.*, 1994) since the reflex response to a push stimulus of up to 3 N has been shown to disappear when local anaesthetic was infiltrated around the stimulated tooth and white noise played into earphones (Brodin *et al.*, 1993b).

The earlier work using the push stimulus could induce SER in about 40% of the experiments using 100 stimuli (Brodin *et al.*, 1993b; Türker *et al.*, 1994). Others,



using a similar push stimulus, could not induce this reflex at all (Bonte, 1993; Louca *et al.*, 1994,1996a). It has been demonstrated in this study that preload and force profile are the main factors that determine the rate of force application which affect the reflex response of the masseter. This study has found several ways to improve the conditions of the push stimulus to induce SER, such as using preload before the actual applied force, or using Type-SSR profile to reduce the fast force component, since fast forces are significantly associated with inducing the inhibitory reflex. With the present optimisation (ie., smooth force profile with preload), this reflex can now be induced in about 60 % of the experiments provided that 100 or more stimuli are used.

Furthermore when the results of several trials in one experimental session were added, the reflex responses became clearer as the size of the prestimulus EMG variations (the size of the error box) became smaller in accordance with the averaging effect. The SER is a more subtle reflex than the inhibitory reflex. Therefore, clear illustration of this reflex requires that the random background fluctuations of the prestimulus EMG be reduced by averaging 100 or more trials.

### **Receptor mechanisms underlying the present results**

It has been established that the majority of periodontal mechanoreceptors can be activated by forces applied both axially and horizontally, and that the direction of stimulation is correctly expressed by *en masse* activity of several receptors (Trulsson *et al.*, 1992). Recording from the inferior alveolar nerve using tungsten electrodes, Trulsson and co-workers (Trulsson *et al.*, 1992; Trulsson, 1993b; Trulsson & Johansson, 1994) have described two different receptor types that were activated by



the mechanical stimulation of the lower front teeth of human subjects. The majority of the receptors (about 80 %) belonged to the 'saturating group' and reached their maximal firing rate under 3 N. These receptors lost their dynamic sensitivities when the preload was above 0.5 N. The second group of receptors was observed less often (about 20 %) and was called 'non-saturating' receptors, which displayed a linear response to force increases up to about 5 N (Trulsson & Johansson, 1994). Unlike the saturating-type receptors, the non-saturating receptors could still be activated by rapidly applied forces (ie., their dynamic sensitivities were not reduced) in the presence of preload (Trulsson, 1993b).

It has been proposed that these two different receptors with varying sensitivities to rate of force application may underlie the reflexes observed in the present study. The apically-situated, slow-rate sensitive, 'low-threshold' receptors (Linden, 1990) may be the ones that have saturating response properties and may also have excitatory connection to the jaw closing muscles. On the other hand, the high-rate sensitive, 'high-threshold' receptors which are situated near the fulcrum (Linden, 1990) may be the 'non-saturating' receptors with inhibitory connections to the jaw closing muscles.

The main response to the 0.5 N preload must come from the saturating afferents since not only do they form the majority of the afferents but they also are very sensitive to forces at this level. The push stimulus that starts from about 0.5 N preload can then activate the saturating and the non-saturating afferents depending on the stimulus intensity and whether or not it has fast components. If the push stimulus of 2.5 N has no fast components, it would stimulate both afferent types. However, the majority of

the response must still come from the saturating afferents since they form 80 % of the afferent population and reach saturation point below 3 N.

If the effective force profile has both slow and fast components, as well as mainly activating the saturating afferents with its slow components, it would stimulate the non-saturating afferents with its fast components since these receptors keep their dynamic sensitivities at high static force levels. Since a 0.5 N preload would diminish the dynamic sensitivity of the saturating afferents while not affecting the dynamic sensitivity of non-saturating afferents (Trulsson, 1993b), the main response to the fast components of the push stimulus should come from the non-saturating afferents.

In conclusion, when the probe induced a smooth push force on the tooth, the dominant response was excitation (SER) since the receptors responding to this stimulus are most likely to be dominated by the saturating afferents. When, however, the push force had fast components, the dominant response probably came from the non-saturating receptors, hence inducing inhibition. In order to illustrate the existence of the sole excitatory pathway that originates from the periodontal mechanoreceptors, it is recommended that the probe should be pressed against the tooth with a force of about 0.5 N before the actual push stimulus starts. The actual push stimulus strength should be about 2.5 N. The stimulating probe should be directed so that it is at a right angle (orthogonal) to the labial surface of the tooth and that during stimulation, it does not slip on the surface of the tooth. The push stimulus should be applied orthogonally to the incisal edge of the labial surface. The shape of the stimulus wave should be smooth and a rubber-tipped probe (Type-SSR) should be used to smooth the force

profile further and prevent slippage. The subject should keep an activity level in his/her ipsilateral masseter of about 10% MVC. The performance of the probe should be monitored on-line using a high-pass filtered (50 Hz) record of the applied force since this approach illustrates fast components of the force should they occur. Reflex responses to at least 100 randomly delivered stimuli should be averaged and quantified using a symmetrical error box.

Different patterns of reflex response to slow and fast stimuli have clear functional significance. During chewing, the mechanoreceptors around the teeth are optimally placed to register the force being applied to the teeth. When the teeth begin to bite slowly into food, the activation of periodontal receptors by the pressure exerted on the teeth can contribute to the level of excitation in the jaw closing muscles (Lavigne *et al.*, 1987; Morimoto *et al.*, 1989; Ottenhoff *et al.*, 1992a,b). This reflex-induced facilitation may help the muscles maintain pieces of food between the upper and lower teeth so that they may then be crushed, or it may, in concert with the muscle spindle reflex control (Lamarre & Lund, 1975), help to overcome unexpected resistances to closing (Ottenhoff *et al.*, 1992a,b). However, when one bites unexpectedly on a rigid object such as a stone in rice while chewing, the resulting brisk forces on the teeth will inhibit the powerful jaw closing muscles (similar to the tap stimulus).

## **CHAPTER 4**

### ***JAW REFLEX RESPONSES TO MECHANICAL STIMULATION OF A HUMAN TOOTH***

#### **4.1 INTRODUCTION**

The reflex connection between the periodontal mechanoreceptors and the jaw closing muscles has been subject to much study. Some of these studies suggested that the reflex responses from these receptors are principally inhibitory (Sessle & Schmitt, 1972; Van Der Glas *et al.*, 1985; Dessem *et al.*, 1988; Bonte *et al.*, 1993; Louca *et al.*, 1996a). In contrast, other researchers have demonstrated evidence for an excitatory connection to the jaw closing muscles from periodontal receptors (Lund & Lamarre, 1973; Amano & Yoneda, 1980; Funakoshi, 1981; Lavigne *et al.*, 1987). Recently, the importance of the rate of rise of the stimulus force in eliciting excitatory or inhibitory responses from the masseter muscle has been addressed. The slowly-rising (push) stimulus induced an excitatory reflex while the rapidly-rising (tap) stimulus usually induced inhibition (Brodin *et al.*, 1993b; Türker *et al.*, 1994). There are also other factors that affect the rate of stimulation force which determine the pattern of reflex response, such as the presence of a preload and the exact stimulus force profile, described in Chapter 3.

In each of these studies, the reflex response was determined using the SEMG of jaw muscles. In contrast to the large number of studies using SEMG, the reflex changes in bite force induced by a tooth stimulus have never been studied systematically

(Yamamura *et al.*, 1993). It is possible that the much-studied masseter SEMG may not represent effective changes in bite force in response to a tooth stimulus. This is because the bite force can be developed using a large number of combinations of activation of jaw muscles or sections of jaw muscles (Van Eijden *et al.*, 1990; Hannam & McMillan, 1994). Hence, the theories regarding the changes in the masticatory forces that originated from reflex studies in only one jaw muscle may be misleading (eg., Brodin *et al.*, 1993b).

Furthermore, there are some difficulties in interpreting the SEMG data. For example, an increase in SEMG, preceding an inhibitory phase, may be an artefact of the averaging process (Widmer & Lund, 1989). Similarly, an increase following a decrease in SEMG records, can simply be a cluster of action potentials delayed by the preceding inhibition (Miles *et al.*, 1987; Bonte & van Steenberghe, 1989). Any such clusters of action potentials related to the stimulus will fire again at about one inter-spike interval and hence induce several peaks and troughs. These changes in the SEMG may be wrongly described as an excitatory connection of the stimulated afferent to the masseter motoneurons (Awiszus *et al.*, 1991; Türker & Cheng, 1994). This is termed a 'synchronisation related error' for the averaged SEMG records (Türker *et al.*, 1999). SEMG also has one other major pitfall, 'number related error' (Türker *et al.*, 1999), where a large postsynaptic potential (PSP) shadows a later PSP, as many of the active motoneurons that discharge in response to the earlier PSP can no longer fire for a further one inter-spike interval (Türker & Powers, 1999). This period resembles a 'silent period' or a period with reduced activity on the averaged graph (Türker *et al.*, 1997).

The bite force, however, is not affected by the above-mentioned artefacts and represents the net response of the masticatory system to mechanical tooth stimulation. Therefore, the present experiments were designed to study the reflex changes in the bite force, in response to a mechanical stimulation of an upper incisor tooth in man. The other aim of this study was to compare the reflex response of the bite force with the SEMG of the masseter in order to see whether SEMG can represent effective changes in bite force in response to a tooth stimulus.

## 4.2 METHODS

Fourteen experiments were carried out on 9 young, healthy and consenting subjects, aged from 19 to 26 years (5 males and 4 females). All subjects had normal dentition and no history of oro-facial neuromuscular dysfunction or orthodontic treatment. These experiments were approved by the Human Ethics Committee of The University of Adelaide.

### 4.2.1 *Experimental Apparatus*

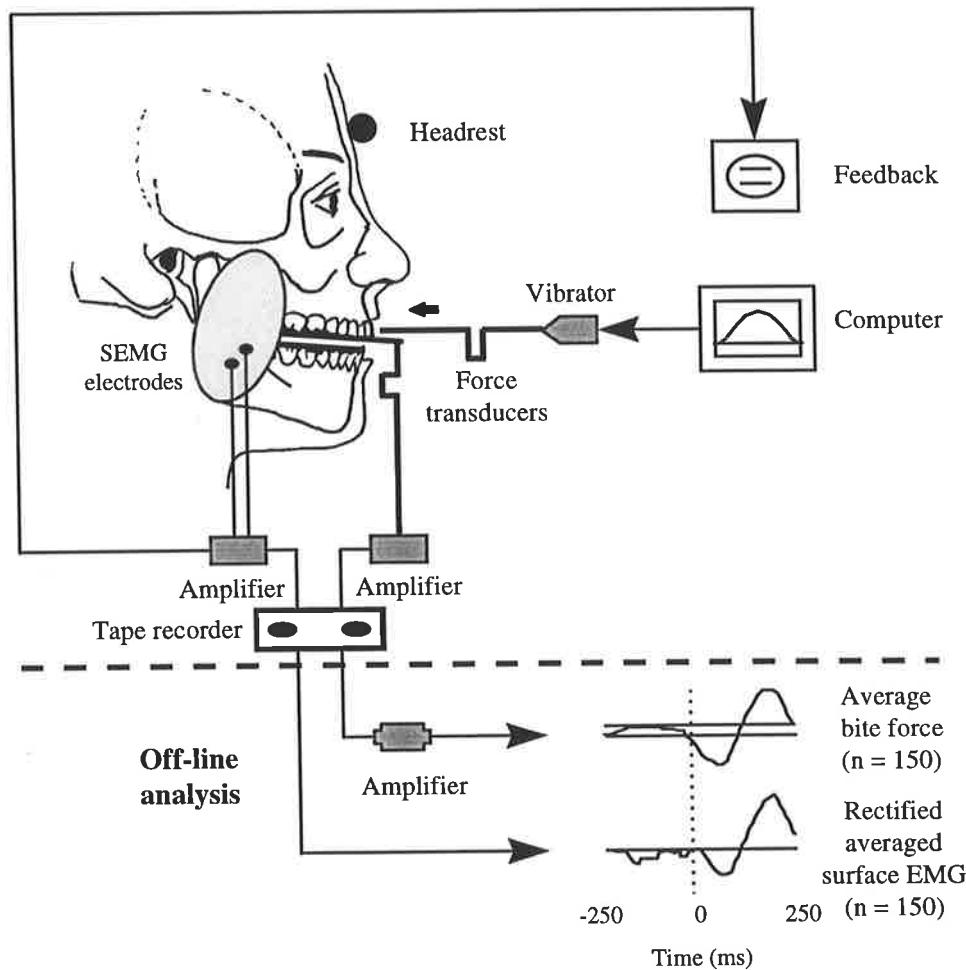
Details of the experimental set-up have been described in Chapter 3 and are summarised here. The subject was seated comfortably with his/her upper teeth held in a fixed relation to a Teflon<sup>®</sup> probe mounted on the moving coil of an electromechanical vibrator. In order to measure the bite force, two bite plates were using instead of one rigid frame described in Chapter 3. During the experiment, the subject was asked to bite into the impression of his/her teeth, which was attached to these two bite plates. The dental impression material was cut away from around the

upper left lateral incisor so that the tooth could be contacted and stimulated by the probe. The position of the head was further secured by a headrest that gently touched the forehead (Figure 4.1).

### ***Periodontal mechanical stimulation***

The periodontal mechanoreceptors of the upper left lateral incisor were stimulated with a 2.5 N slowly-rising force (25 N/s) or a rapidly-rising force (1250 N/s). The stimulus was delivered orthogonally to the labial surface of the tooth with the inter-stimulus interval varying randomly between 2-5 s. For the slowly-rising force, the shape of the force profile was a slowly-rising half sinusoid wave (time to peak = 100 ms; Type-SSR force profile in Chapter 3). The probe applied a preload (0.2 - 0.5 N) to the tooth preceding the stimulus, and the incisal edge rather than the centre of the tooth was stimulated. To make sure that the probe did not slip and that the effective stimulus profile was smooth, the high-pass filtered force (at 50 Hz) record was carefully monitored throughout the experiment.



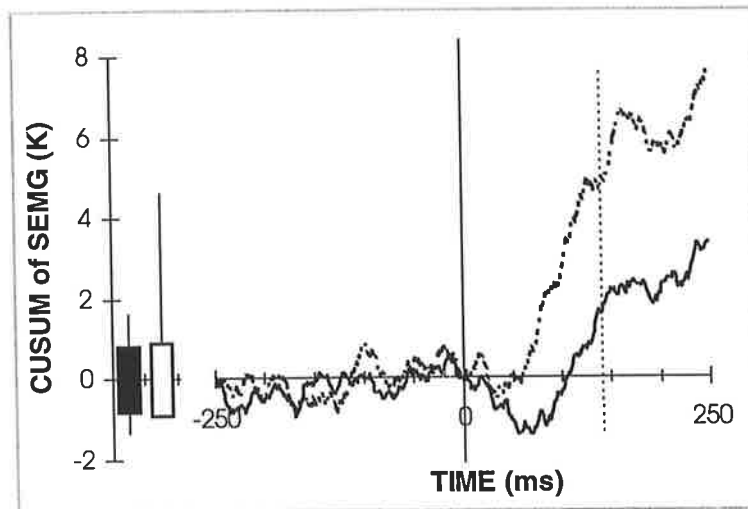


**Figure 4.1 Diagrammatic illustration of experimental apparatus**

The shape of the stimulus profile was determined on a computer, which sent this wave to a vibrator to stimulate the tooth. The strength and the profile of the stimulus were measured by a force transducer placed in series with vibrator. The bite force was measured with a force transducer mounted under the upper bite plate, which carried the impression of the upper teeth. The lower bite plate that carried the impression of the lower teeth was fixed to the rigid frame to ensure that the vertical distance (5 mm) between the bite plates was kept constant. The position of the subject's head was further secured by asking the subject to rest his/her forehead on a horizontal bar (headrest). The subject controlled the level of muscle activity with the help of feedback from the ipsilateral masseter. The SEMG of both masseters and the isometric bite force were recorded simultaneously. During the off-line analysis, the bite force was amplified, filtered and averaged in 150 trials. The SEMG was rectified and averaged (150 trials) around the time of the stimulus.

### ***Surface EMG***

Bipolar surface electrodes were placed over the masseter on both sides of the face. The SEMG signal was filtered (20-1000 Hz), amplified (2,000 times) and recorded on a video recorder (Vetter 400) for off-line analysis. During off-line analyses, the SEMG was filtered (20-500 Hz), sampled at 1 kHz, full-wave rectified and averaged (12 bits resolution) around the time of the stimulus. The CUSUM of the averaged SEMG record was constructed (1 ms bin-width). The procedure performed to obtain reflex response from the CUSUM was fully described in Chapter 3 and is summarised here. From the prestimulus period of the CUSUM records of SEMG, the maximal positive and negative deflections of the SEMG in the prestimulus period were obtained. The larger of the two values was then used to make a symmetrical 'error box' (Figure 4.2, ■ for ipsilateral & □ for contralateral masseter). From the CUSUM records, the existence of a reflex response was determined by comparing the size of the error box with the deflections in the poststimulus CUSUM, within the reaction time to this stimulus. The reaction time for the masseter SEMG for slowly- and rapidly-rising stimuli has been reported to be 140 and 80 ms respectively (Brodin *et al.*, 1993a). Any response above or below the limits of the error box occurring before the reaction time was considered as a significant increase or decrease in SEMG, respectively (Figure 4.2).



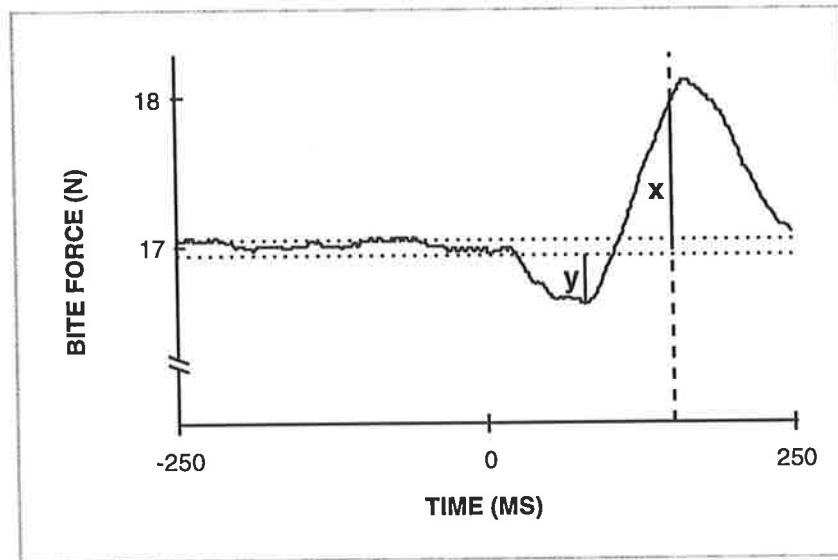
**Figure 4.2 Reflex responses of bilateral masseter muscles to slowly-rising stimulation**

The reflex response was determined by comparing the size of error boxes (■ & □) with the deflections of poststimulus CUSUM within 140 ms of the stimulus (the SEMG reaction time to this stimulus). The bars on the error boxes represent the maximal reflex change occurring before reaction time. Ipsilateral masseter CUSUM is illustrated as a solid line which shows a decrease followed by an increase. The broken line indicates the reflex response from the contralateral masseter which shows only an increase. The reaction time (140 ms) is indicated by a vertical dotted line and the stimulus was given at time zero. ■ = maximal prestimulus variability in the ipsilateral masseter SEMG. □ = maximal prestimulus variability for the contralateral masseter SEMG.

***Bite force***

The isometric bite force was measured by a strain gauge (Load Cell - A&D Co., Ltd. LC1205-K100; sensitivity: 0.005Kg to 100Kg) mounted on the upper bite plate. The minimum bite force that could be measured reliably was 50 mN. This limitation is not expected to affect the bite force values in this study since the steps of bite force reported here are in 100 mN. The bite force was recorded on a video recorder for off-line analysis.

During the off-line analyses, the bite force signal was amplified, filtered (DC - 50 Hz), and averaged over 150 trials. The net reflex change in the closing force in response to the tooth stimulation was determined by using the bite force as the source and the timing of the stimulus as the trigger in an averaging program. From the averaged records of the bite force, the maximal variation of the prestimulus (-250 to 0 ms) force was measured. In the poststimulus period, an increase of the bite force that was above, or a decrease of the bite force that was below the maximal prestimulus force variation, was determined (Figure 4.3).



**Figure 4.3** Reflex response of bite force in response to a slowly-rising stimulus

The maximal variability in the averaged prestimulus bite force is shown by two horizontal dotted lines. The changes in the bite force which were above or below these lines and occurring before the reaction time (vertical dashed line at time 155 ms) were determined as a reflex response. The ratio of the maximum to minimum reflex response in bite force was calculated. **Ratio** =  $x / y$  where  $x$  is the maximal increase and  $y$  is the maximal decrease in the poststimulus period before the reaction time.

It has been reported earlier (Brodin *et al.*, 1993a) that the fastest reaction time to slowly- and rapidly-rising mechanical stimuli to teeth as expressed on the masseter SEMG were 140 and 80 ms respectively. However, due to the time taken for the electrical events to induce measurable force changes, the reaction time for the force record needed to be corrected accordingly with the SEMG-force relationship observed for the masseter muscle in human subjects. The SEMG-force delay was found to be 15 ms in this study, which is similar to the findings of others in hand muscles (Johansson & Westling, 1984). Therefore the corrected reaction time for the force record was 15 ms longer than the reaction time for the SEMG, that is, 155 ms for the slowly-rising and 95 ms for the rapidly-rising stimuli.

With slowly-rising stimuli, the ratio of the maximal increase to the maximal decrease in the bite force was calculated as: **ratio** = **x** / **y**, where **x** was the maximal increase and **y** was the maximal decrease in the bite force preceding the reaction time (Figure 4.3).

#### **4.2.2 Experimental Protocol**

The subjects were divided into two groups (A and B):

In group A, 8 experiments were carried out on 6 subjects. In each experiment, the subject bit into bite plates on which a strain gauge was mounted to measure the bite force: the inter-incisal distance was 5 mm. The subject was asked to control the activity of the ipsilateral masseter muscle using the discharge frequency of one of its motor units (Miles & Türker, 1986). To record and discriminate SMU, a fine bipolar electrode (Teflon<sup>®</sup>-insulated, 0.07 mm diameter silver wire) was inserted to record

motor unit potentials from the left masseter muscle. The shapes of motor unit potentials were recognised using a computer-based template-matching algorithm (SPS-8701). Using the discharge frequency of a selected unit as feedback, the subject was asked to maintain a contraction level that discharged the unit at about 12 Hz. The slowly-rising stimuli were applied when the unit was discharging regularly. In order to avoid muscle fatigue, each run consisted of 50 stimuli. The subject was asked to rest on the bars for 30 seconds between each run, and then repeat the biting for the other two runs. After 150 stimuli (3 runs), the subject was asked to come off the bite plates and rest for 10 minutes, thus completing one experimental session. In each experiment, 4 - 6 experimental sessions of 150 stimuli were performed. Therefore, 35 experimental sessions were achieved in 6 subjects in 8 separate experiments. Each experimental session used a different motor unit as the feedback.

In order to compare the reflex effect on bite force by rapidly- and slowly-rising applied force, in group B, 6 subjects participated in 6 experiments in which the reflex response of bite force was studied by using slowly-rising and rapidly-rising stimuli. Using 5% and 10% of the maximal SEMG activity as the feedback, 2.5 N slowly-rising or rapidly-rising stimuli were applied to the tooth. In each experiment, 4 experimental sessions, 2 for slowly-rising and 2 for rapidly-rising stimulus, of 150 stimuli were achieved.

### 4.2.3 Statistical Analyses

Comparisons of the reflex response patterns to different stimuli (slowly-rising and rapidly-rising) and to different methods (SEMG and bite force) were determined using contingency table analysis (Everitt, 1993). The Spearman rank order correlation coefficient rho (Pagano, 1994a) was calculated from the bite force ratio against the background bite force level. A *P*-value of less than 0.05 was considered statistically significant for all tests.

## 4.3 RESULTS

### 4.3.1 The Reflex Response Patterns Produced by Slowly-Rising Stimulation

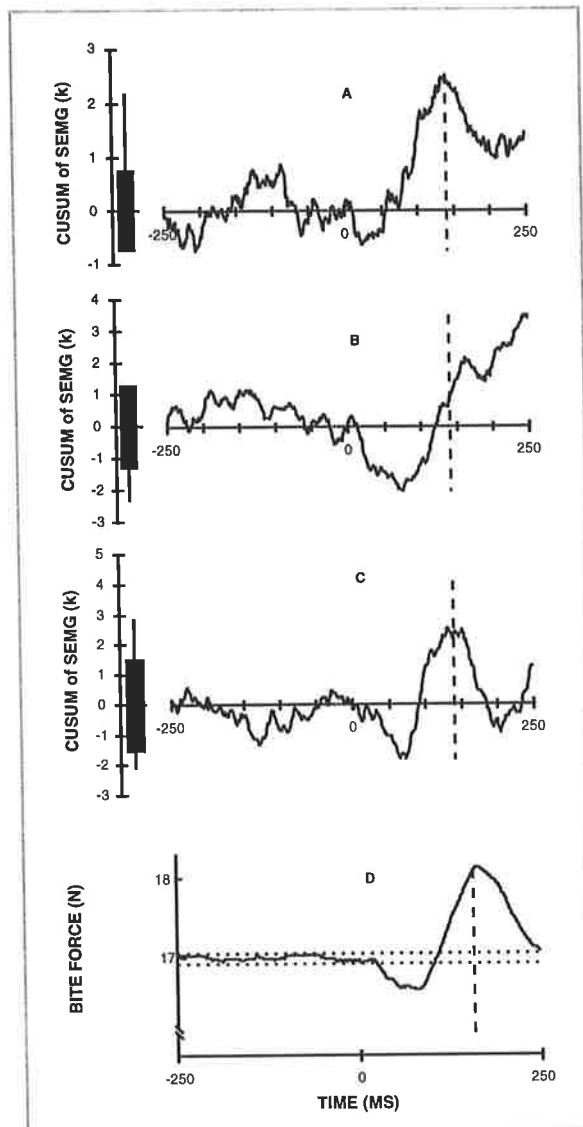
The reflex response pattern of Group A, determined by the CUSUM of the SEMG and bite force, is illustrated in Table 4.1. Since the ipsi- and contralateral masseter demonstrated very similar reflex responses in the SEMG (Bonte *et al.*, 1993; also see Chapter 3), the SEMG results described below came from the ipsilateral side only. Using the SEMG analysis method, 18 out of 35 experimental session records showed a sole excitatory reflex response (SER); 6 out of 35 were sole inhibition (SIR); 8 out of 35 were inhibition followed by excitation (IER) and 3 out of 35 were No reflex (that is, poststimulus CUSUM deflection did not go above or below the limits of the error box). The main pattern in the SEMG was SER. Conversely, in the averaged bite force records, the dominant reflex pattern was IER, which is a decrease followed by an increase in the net bite force (Table 4.1 and Figure 4.4).



**Table 4.1 Reflex responses of SEMG and bite force to a slowly-rising stimulus**

	SER	SIR	IER	No Reflex	Total
SEMG	18	6	8	3	35
Bite Force	0	4	31	0	35

With slowly-rising stimulation, the incidence of reflex response patterns in the SEMG of ipsilateral masseter and the bite force in 35 experimental sessions are shown. SER= sole excitatory reflex response or an increase in bite force; SIR= sole inhibitory reflex response or a decrease in bite force; IER= inhibition followed by excitation or a bite force decrease followed by an increase. The reflex response pattern in the SEMG was compared with the reflex response pattern in the bite force. The patterns were significantly different ( $P < 0.001$ ).



**Figure 4.4 Reflex patterns of SEMG and bite force to a slowly-rising stimulus**

The CUSUM of the SEMG records displayed three different reflex patterns in response to slowly-rising stimulation: sole excitation (**A**), sole inhibition (**B**), inhibition followed by excitation (**C**). There was mainly one dominant reflex response pattern for the bite force, a bite force decrease followed by an increase (**D**). The CUSUM in **A** and the bite force in **D** were recorded simultaneously from one subject. **B** and **C** came from two other subjects. Stimulation was given at time zero. The vertical dashed line indicates the reaction time of the subject to this stimulus (140 ms in CUSUM, 155 ms in bite force). Any change in the records that occurred before the reaction time which was larger than the prestimulus limits was considered a reflex response.

The reflex response pattern and the average bite force for each trial, is illustrated in Table 4.2.

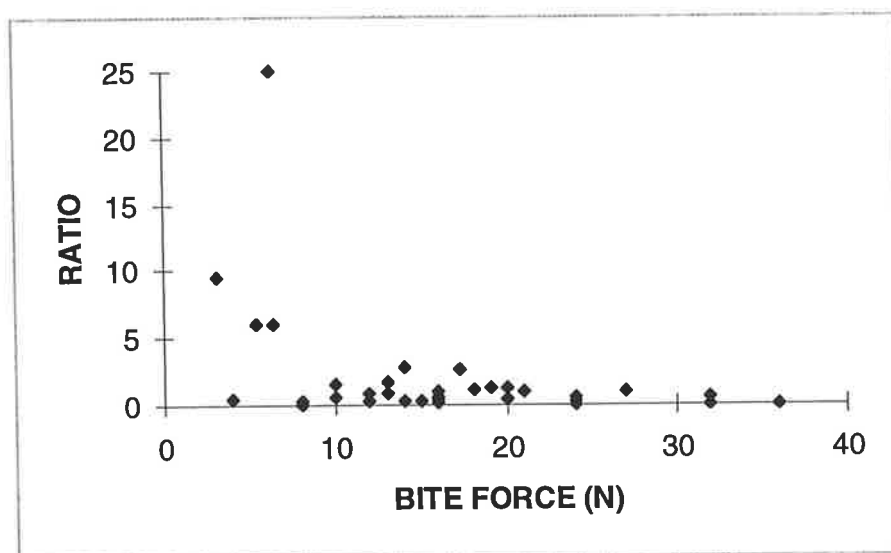
**Table 4.2 Individual reflex responses of SEMG and bite force**

Subjects	Trials					
	1	2	3	4	5	6
Se	IER (8.0)	SER (8.0)	SIR (8.0)	SER (24.0)		
C	SER (3.2)	SER (4.0)	SER (15.0)	IER (32.0)		
T	SER (5.4)	SER (6.4)	IER (6.4)	SER (17.2)		
M	SIR (10.0)	SIR (13.0)	SIR (18.0)	SIR (24.0)		
Si	SER (16.0)	SER (16.0)	IER (20.0)	SER (32.0)		
C	SER (13.0)	SER (16.0)	SER (16.0)	0 (27.0)	0 (36.0)	
H	SIR (10.0)	0 (12.0)	SER (12.0)	SER (24.0)		
T	IER (14.0)	IER (14.0)	SER (15.0)	SER (19.0)	IER (20.0)	IER (21.0)

At least four trials were completed for each subject. Two of the subjects (T and C) participated on two experimental days. The reflex responses are indicated for each trial in each subject. The average force level (in Newtons) for each trial is shown in parenthesis. SER= sole excitatory reflex response; SIR= sole inhibitory reflex response; IER= inhibition followed by excitation; 0= no reflex response.

In the poststimulus period, the CUSUM of the SEMG, going beyond the error box size was extrapolated back to the baseline, and the timing of the first deflection in the same direction was noted as the latency of the reflex response (see Chapter 3). The average latency for the inhibitory reflex response in the SEMG was 20 ms (ranging from 8 ms to 33 ms) and the bite force decreased with an onset latency of about 35 ms (ranging from 23 ms to 74 ms). For the bite force records, the latency of the reflex response was measured from the point of deflection below or above the prestimulus variability limits. The average onset latency difference between the SEMG and the bite force was 15 ms. This delay between the muscle's electrical activity and the resultant force change was similar to that found in hand muscles (8-12 ms, Johansson & Westling, 1984).

To find out how background clenching levels affected the reflex response of the bite force, the ratios of the increase/reduction of the bite force were calculated (Figure 4.3). The ratio data from 6 subjects were then plotted against the background bite force (Figure 4.5). There was a significant negative relationship between the ratio and the background bite force ( $\rho = -0.3825$ ,  $P < 0.05$ ). When the background bite force was low, the ratio was high. Conversely, when the background bite force was high, the ratio was low, almost reaching zero.



**Figure 4.5** The ratio of the reflex bite force increase and decrease vs the background bite force level

The ratios of reflex bite force changes to slowly-rising stimulation against the background clenching levels in Newtons (N) are shown. The data came from 35 experimental sessions in 8 separate experiments which were performed on 6 subjects. When the background bite force increased, the ratio decreased ( $\rho = -0.3825$ ,  $P < 0.05$ ).

### 4.3.2 The Reflex Response Evoked by Slowly-rising and Rapidly-rising Stimuli

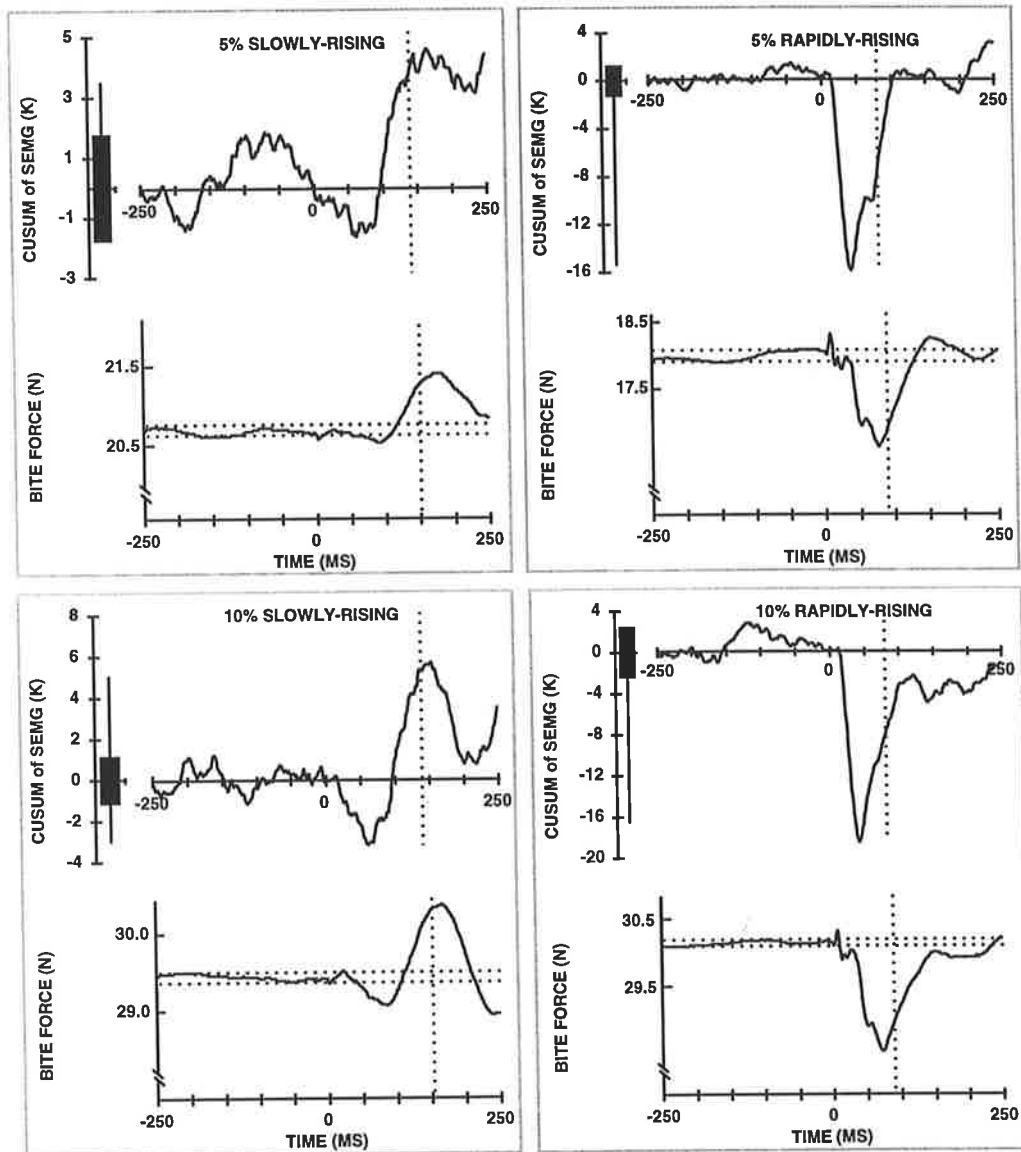
Six subjects participated in 6 experiments at two different bite force levels (5% and 10% MVC) using rapidly-rising and slowly-rising stimuli. The SEMG and bite force reflex patterns in response to rapidly-rising stimulation are summarised in Table 4.3. In the SEMG records, the rapidly-rising stimulus induced three different reflex response patterns (SER, SIR, IER). However, in the bite force records, there was only one reflex response pattern; a net reduction in the closing force (SIR). The reflex response patterns for the SEMG, and those for the bite force, were significantly different.

**Table 4.3 SEMG and bite force in response to a rapidly-rising stimulus**

	SER	SIR	IER	No Reflex	Total
SEMG	1	4	7	0	12
Bite Force	0	12	0	0	12

The reflex pattern of the SEMG and the bite force in response to rapidly-rising stimulation is illustrated in 12 experimental records on 6 subjects. There were 3 different reflex response patterns in the SEMG records (SER, SIR, IER) and only one reflex response pattern in the bite force records (SIR). The pattern in the two rows were significantly different.

Different reflex responses produced by slowly-rising and rapidly-rising stimulus are demonstrated in Figure 4.6.



**Figure 4.6** Different reflex responses produced by slowly-rising and rapidly-rising stimulus

This figure shows a sample of different reflex responses in the SEMG and its corresponding bite force produced by slowly-rising and rapidly-rising stimuli. In the left panel, the reflex responses in the CUSUM of the SEMG and the bite force evoked by slowly-rising stimulus are shown. In the right panel, the reflex changes in the CUSUM of the SEMG and the bite force which were elicited by rapidly-rising stimuli are shown. The background clenching level of the top two records were 5% of the maximum activity of ipsilateral masseter SEMG and the bottom two were 10% of the maximum SEMG activity. The reaction time is indicated by a dotted line (140 ms for CUSUM and 155 ms for bite force with slowly-rising stimulation; 80 ms for CUSUM and 95 ms for bite force with rapidly-rising stimuli).

The incidence of reflex patterns in bite force, in response to slowly-rising or rapidly-rising stimulation, is shown in Table 4.4. For the slowly-rising force, the reflex response patterns in the bite force were: SER, SIR, and IER, but mainly IER as observed in Group A. With the rapidly-rising stimulus, however, the reflex response in the bite force was only a net force decrease (SIR). The difference between the reflex response patterns in the bite force evoked by slowly-rising and rapidly-rising stimulation was significantly different.

**Table 4.4 Reflex responses of the bite force to slowly- and rapidly-rising stimuli**

	SER	SIR	IER	No Reflex	Total
Slowly-rising	2	2	8	0	12
Rapidly-rising	0	12	0	0	12

The reflex response patterns in the averaged bite force in response to slowly- and rapidly-rising stimuli are shown. For the slowly-rising stimulus, the dominant reflex response pattern was IER. With the rapidly-rising stimulus, there was only one reflex response pattern (SIR). The patterns of reflex response elicited by two stimulus types were significant different.



## 4.4 DISCUSSION

This study showed, for the first time, that the net response of all jaw muscles to a mechanical tooth stimulus depends on the rate of rise of the stimulus force. While the rapidly-rising stimuli always induced a net decrease in the bite force, slowly-rising stimuli mainly induced a small decrease followed by an increase. The simultaneously-recorded SEMG of the ipsilateral masseter muscle, on the other hand, displayed three different combinations of reflex responses. Therefore, the reflex change in the masseter muscle does not represent the net reflex responses of all jaw muscles in response to a mechanical tooth stimulus and that the reflex response to slowly-rising stimulation varies with the background level of bite force.

### 4.4.1 *Reflex Responses of SEMG of the Masseter and the Bite Force*

Although the masseteric SEMG may represent the closing force in the anaesthetised rabbit (Hidaka *et al.*, 1997), human studies do not indicate such a close relationship between the masseteric SEMG and the bite force (Mackenna & Türker, 1983). Despite that, the reflex response in the masseter SEMG has often been taken to represent the bite force, and speculations have been made regarding changes in bite force during chewing (eg. Brodin *et al.*, 1993b).

It is well known that bite force is developed by at least three pairs of major jaw closing muscles and also opposed by several jaw-openers. Not only do individual jaw muscles have preferred functions, but also they even have functional compartments, which are preferentially activated in certain tasks (Van Eijden *et al.*, 1990; Hannam & McMillan,

1994). The complexity of jaw muscles is further displayed by their histochemical and neurological differences from the limb muscles (Lund, 1991). The jaw opening muscles lack muscle spindles and do not show marked reflex responses in human subjects (Matthews, 1975). However, they still affect the bite force by stiffening the jaw, especially during difficult or novel tasks (Miles & Madigan, 1983). Therefore, both the jaw-openers and jaw closing muscles may take part in determining the net output of the masticatory system to a mechanical tooth stimulus.

Before the present study, the net reflex response to mechanical stimulation of a tooth was rarely studied (Yemm, 1972a,b; Türker & Miles, 1985; Yamamura *et al.*, 1993). In the only similar study, the reflex response was assumed to continue for three seconds after the application of the stimulus (Yamamura *et al.*, 1993). This is more than 20 times the reaction time that has been measured in response to a similar stimulus to a tooth (Brodin *et al.*, 1993a). Therefore, Yamamura's work cannot be compared with the reflex results described here.

A comparison of the reflex response of the SEMG of the masseter and the bite force in response to slowly-rising stimulation (Table 4.1) shows that the main pattern in the bite force was IER, a net bite force decrease followed by an increase. However, sole excitation (SER) was the dominant pattern observed in the SEMG of the masseter recorded simultaneously (Figure 4.4). One possible explanation for these somewhat conflicting results could be that other jaw muscles, such as the jaw opening muscles, contributed to the decrease of bite force. Secondly, the method of analysis (see Chapter 3) may cause this difference. In the SEMG analysis method used, short-lasting

reflex responses could be masked by the size of the error box. Therefore, the existence of such reflex responses can be underestimated. The differences in the reflex patterns between the masseteric SEMG and the bite force indicate that either the reflex response pattern of masseter does not represent the net response of all jaw muscles, or the SEMG analysis methods used are not capable of recording all reflex responses.

#### ***4.4.2 Receptors and Pathways***

In reduced animal preparations, it is possible to induce activity in two different pathways in response to tooth stimulation. One is presumably the disynaptic inhibitory pathway which responds to rapidly applied mechanical stimuli, and the other is the longer-latency excitatory response, when the rate of application of the stimulus is low (Kidokoro *et al.*, 1968a,b; Dessem *et al.*, 1988; Appenteng *et al.*, 1989; Linden, 1990).

In human subjects, the periodontal mechanoreceptors can be activated by forces applied in many directions (Trulsson *et al.*, 1992). Therefore, the direction of stimulation used in the present experiments must have activated a large number of periodontal mechanoreceptors. The majority of the receptors belong to the 'hyperbolic' group which reach their maximal firing rate under 3 N and lose their dynamic sensitivities when the preload is above 0.5 N. The second group of receptors is known as 'nearly-linear' receptors and is observed less often. These receptors display a nearly-linear response to force increases of up to about 5 N (Trulsson & Johansson, 1994). Unlike the hyperbolic receptors, the nearly-linear receptors do not lose their dynamic sensitivities in the presence of preload (Trulsson & Johansson,

1994,1995). It is more likely that these two different receptors with varying thresholds and sensitivities to the rate of force application (see Linden, 1990 for similar classification in periodontal mechanoreceptors in animals) may underlie the reflexes observed in the present study.

However, it is possible that no matter how carefully the stimulus force is applied on the tooth, it can activate both of these reflex pathways simultaneously. If the force is applied slowly, the excitation may dominate inhibition. However, if the force is applied rapidly, and the fast component of the force is very large, a large group of rapidly adapting receptors will be activated (Trulsson & Johansson, 1994) and an inhibitory reflex response would be dominant.

It could be argued that the initial reduction of the bite force causes sudden jaw-opening by releasing the constant pressure from the bite plates and allows the bars to recoil. This opening 'stimulus' may stretch the muscle spindles in the jaw closing muscles, which may then result in a reflex increase of the bite force (Yemm, 1972a,b; Mitchell *et al.*, 1992). However, this increase in bite force was only observed in response to the slowly-rising stimuli. If the initial reduction of bite force was the stimulus for the late increase in force, then, there should have been a larger increase in bite force in response to the rapidly-rising stimuli which induced a much larger reduction in bite force than did the slowly-rising stimuli. It has been tempting to conclude therefore that the muscle spindles may not contribute to the increase in the bite force in response to slowly-rising stimuli.

#### ***4.4.3 The Reflex Pattern of the Bite Force vs the Background Clenching Level***

In the present study, it became clear that using slowly-rising stimulus, the reflex response varied, depending on the background bite force level. At the low bite force level, there was a small reduction, followed by a relatively large increase in the bite force. On the other hand, when the background bite force level was high, the reflex reduction in bite force became dominant. There are at least two possible explanations for this phenomenon.

Firstly, the differences in reflex response may be due to the recruitment of different types of motor units by the same periodontal stimulus. For example, it has been reported that, in the masseter of the lightly anaesthetised rat, small and large motor units receive excitatory and inhibitory reflex responses from the periodontal mechanoreceptors, respectively (Yamamura & Shimada, 1992). Therefore, at a low bite force level, the reflex excitation of the small-sized motor units would be relatively large and would help to hold food firmly and manipulate it between the teeth (Trulsson & Johansson, 1995). However, when the background bite force level is already high, the same periodontal input may inhibit the larger motor units that are operating at that level of bite force, thereby limiting further increase in bite force to protect the teeth and supporting tissues from damaging forces. This would mean that large forces cannot be developed reflexly and that the reflex increase in the bite force is somewhat limited to the forces encountered in the preceding chewing cycle (Van Der Bilt *et al.*, 1995).

This system would work quite well, in that each time a large force is required to overcome an unexpected resistance, the force increase would involve the higher centres and control its damaging effects (Ottenhoff *et al.*, 1992b). Conscious interference in bite force can also increase the flow of information from the receptors to the cortex, which is reduced during normal chewing (Olsson *et al.*, 1986; Lund, 1991), giving the cortex precise information about bite performance.

Secondly, it is possible that a presynaptic effect could modify the efficacy of the synaptic input of the periodontal mechanoreceptors to the motoneurons of jaw muscles. Such presynaptic modulation on the primary afferent input has been well recognised during mastication (Lund & Olsson, 1983; Olsson *et al.*, 1986; Van Der Bilt *et al.*, 1997). This modulation is geared to limit the forces developed by reflex connections of the primary afferents to the motoneurons. In this case, at higher bite force levels, the peripheral and central input to the interneuronal system that control the efficacy of the synaptic input on the motoneurons would also be high. This extra input could then induce presynaptic modulation on the periodontal mechanoreceptor input on the motoneurons.

Furthermore, the effective reflex mechanism of the masticatory muscles, as indicated by the averaged bite force records, shows that it might protect the teeth and supporting tissues from damaging forces when the applied stimulus is developing rapidly, such as when biting on a small stone in food. After a brief inhibitory period, it might also help increase the bite force to hold the food between the teeth if the change in stimulus rate is slow, such as when biting on a piece of meat, especially at low bite force levels.

It is not possible to deduce that these reflexes will work in exactly the same way during natural mastication. It is well known that the effectiveness of the primary afferent input is under presynaptic and postsynaptic modulation during mastication (Olsson *et al.*, 1986; Van Der Bilt *et al.*, 1997). However, some circumstantial evidence from key publications in this field indicates that the reflex mechanism, as described in this study, may be functioning under cortically-induced chewing in the anaesthetised rabbit (Lavigne *et al.*, 1987; Morimoto *et al.*, 1989). Chewing on steel balls caused a jaw-opening reflex as an initial response (Figure 8 of Lavigne *et al.* 1987). The jaw-opening reflex did not occur in subsequent cycles, instead, the periodontal input induced excitation rather than inhibition in the jaw closing muscles. This adaptation may have occurred by reducing the effectiveness of the periodontal input and increasing the effectiveness of the muscle spindle input to the motoneuron pool (Van Der Bilt *et al.*, 1997). This initial jaw-opening reflex response was not observed when the obstruction was a foam strip (Figure 3 of Morimoto *et al.* 1989). In these experiments, a steel ball between the teeth may be compared to the rapidly-rising stimulus and the foam to the slowly-rising stimulus used in current study.

**CHAPTER 5**

***REFLEX RESPONSES OF SINGLE MOTOR UNITS***

***IN HUMAN MASSETER TO SLOWLY-RISING***

***STIMULATION OF A TOOTH***

**5.1 INTRODUCTION**

The presence of excitatory reflex responses in jaw closing muscles to mechanical tooth stimulation is controversial. This may be due to the fact that there is no general agreement regarding the stimulus used to elicit the reflex response. Another possible reason for this controversy may be found in the method of recording the reflex response. Most studies have utilised SEMG to represent the muscle activity (see Chapter 3). However, interpreting the SEMG can be misleading (see Chapter 4), as it is often not possible to determine whether the peaks and troughs represent excitation or inhibition of the underlying motoneuron pool (Widmer & Lund, 1989). Additionally, SEMG can give no information about the behaviour of individual motor units. High threshold (large-sized) and low threshold (small-sized) motor units may respond differently to a stimulus (Garnett & Stephens, 1981), but this can not be detected using SEMG.

Although the size principle has been demonstrated in many animals (Henneman & Olson, 1965; Henneman *et al.*, 1965a,b; Mendell & Henneman, 1971; Clamann *et al.*, 1974a; Henneman & Mendell, 1981; Bawa *et al.*, 1984) and humans (Milner-Brown *et*



*al.*, 1973b,c; Yemm, 1976,1977; Stålberg *et al.*, 1986; Stålberg, 1986; Jones *et al.*, 1994), deviations from the normal recruitment order have been shown to occur when the motoneuron pool is the target of certain peripheral inputs (Burke *et al.*, 1970; Garnett & Stephens, 1980,1981) or during controlled eccentric contractions (Nardone & Schieppati, 1988; Nardone *et al.*, 1989). For example, several studies have demonstrated that cutaneous input to the motoneuron pool disrupts the normal order of recruitment, since the synaptic input from cutaneous receptors can affect different-sized SMUs selectively, favouring the recruitment of the large motoneurons and inhibiting the small motoneurons (Kanda *et al.*, 1977; Garnett & Stephens, 1980,1981; Kanda & Desmedt, 1983). This response is important in ballistic movements, or actions requiring rapid alteration of agonist and antagonists (Smith *et al.*, 1980), or in skilled manipulatory activities of the human hand (Garnett & Stephens, 1980; Kanda & Desmedt, 1983). Within the tooth-supporting tissues, the periodontal mechanoreceptors are very similar to the cutaneous mechanoreceptors histologically and physiologically (Linden *et al.*, 1994,1995). Their activity corresponds directly to the amount and rate of force applied to the tooth (Linden, 1990; Hannam & McMillan, 1994; Trulsson & Johansson, 1994; Linden *et al.*, 1995). The similarities between the two receptor systems leads to the hypothesis that the periodontal mechanoreceptor input to the motoneurons of jaw muscles may also be distributed differentially.

In this study, the effect of periodontal mechanoreceptor stimulation using slowly-rising forces on simultaneously-recorded human masseter SMUs of different sizes is reported. Slowly-rising forces were preferred in this study since they are more likely to stimulate only the periodontal mechanoreceptors (see Chapter 3,4)

## 5.2 METHODS

Experiments were carried out on 7 healthy, consenting volunteers aged from 19-22 years. All subjects had normal dentition with no history of oro-facial neuromuscular dysfunction or orthodontic treatment. The experiments were approved by the Human Ethics Committee of The University of Adelaide.

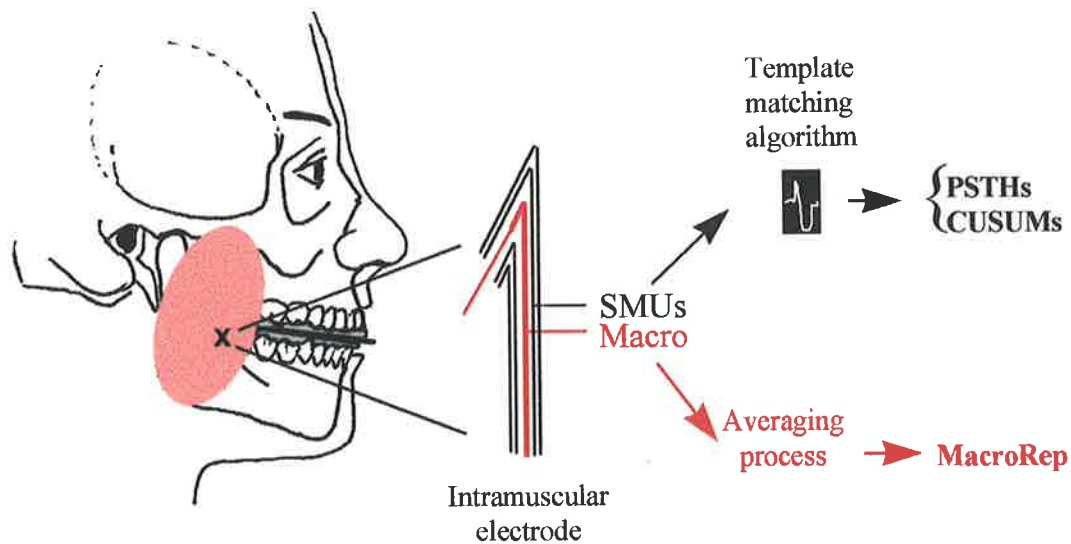
### 5.2.1 *Periodontal Stimulation*

The parameters of the periodontal mechanical stimulation are fully described in Chapter 3. Briefly, using a stimulating probe attached to a mechanical vibrator, a 0.5 N static (preload) and a 2.5 N dynamic (push) force were applied orthogonally to the labial surface of the upper left lateral incisor tooth. The push force followed a slowly-rising half sinusoidal profile (Type-SSR) and reached the total force of 3.0 N in 100 ms. The inter-stimulus interval varied randomly between 2 and 5 seconds.

### 5.2.2 *Single Motor Unit Recording*

Five Teflon<sup>®</sup>-insulated silver wires (AG3T, bare diameter 76µm, Medwire Corp.) were inserted into the anterior deep portion of the left masseter to a depth of about 2 cm using a 23G needle (Figure 5.1). The needle was then withdrawn, leaving all the wires in the belly of the muscle. The insulation material was stripped from the terminal 15 mm of one of the wires to produce a Macro EMG electrode. The Macro EMG activity was recorded using the Macro EMG electrode within the muscle against an ear clip electrode placed on the opposite ear lobe. The other four wires were

completely insulated except for the tip. They were divided into two pairs, each pair forming a bipolar SMU electrode. The tips of the pairs were located along the stripped part of the Macro EMG electrode, separated from each other by about 3 mm. The Macro EMG and SMU wires were bent over to form hooks (see Figure 5.1). This approach ensured optimal contribution of all recorded SMUs, to the Macro EMG (Stålberg, 1980). The SMU signals were filtered at 500 Hz-5 kHz and the Macro EMG at 50 Hz-5 kHz. All signals were recorded on a video tape (Vetter 400) for off-line analysis. The isometric bite force was measured by a strain gauge mounted on the upper bite plates (see Chapter 4, Figure 4.1) and was also recorded for off-line analysis.



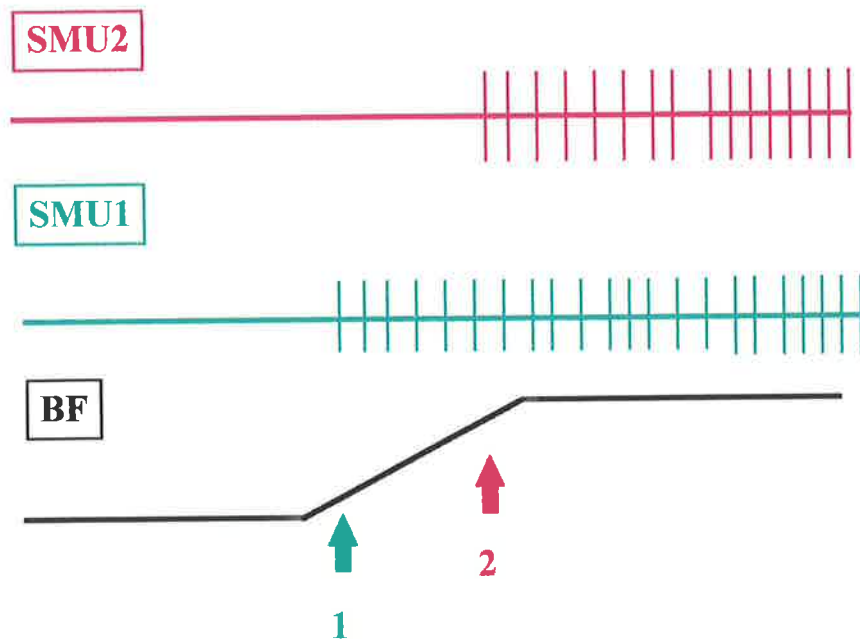
**Figure 5.1 Diagrammatic illustration of the recording of single motor unit activity**

One Macro EMG and two pairs of bipolar single motor unit electrodes were inserted into the deep anterior part of left masseter muscle. The signal from the single motor units was analysed using a template matching algorithm and the probabilities of single motor unit firing were demonstrated by the peristimulus time histogram (PSTHs) and the CUSUMs of PSTHs. The Macro EMG signal was averaged to determine the representation of the single motor unit in the Macro EMG (MacroRep).

### 5.2.3 *Experimental Protocol*

The details of the experimental set-up are given in Chapter 3 and summarised briefly here. The subject bit into an impression of his/her upper and lower teeth which was mounted on bite plates, with a vertical separation of 5 mm. The impression material was cut away from around the upper left lateral incisor so that the tooth could be reached and stimulated by a probe.

In each experimental run, the subject was asked to maintain a contraction of the masseter so that at least one SMU in each electrode fired regularly. The subject was aided in this task by audio and visual feedback of the discharge frequency of one of the units. When the feedback unit was discharging regularly, the periodontal stimulation was delivered to the tooth. In each experimental run, 50 identical stimuli were delivered. The experimental run was repeated up to three times with the same parameters to make up an experimental session. Between each run, the subject was asked to rest on the bite plates for 30 seconds and after 3 runs (1 experimental session) were complete, the subject performed a slow isometric force ramp contraction of 40 N in 15 seconds, to determine the force recruitment threshold of the motor units identified in that experimental session (Figure 5.2).



**Figure 5.2 Measurement of force recruitment threshold of single motor units**

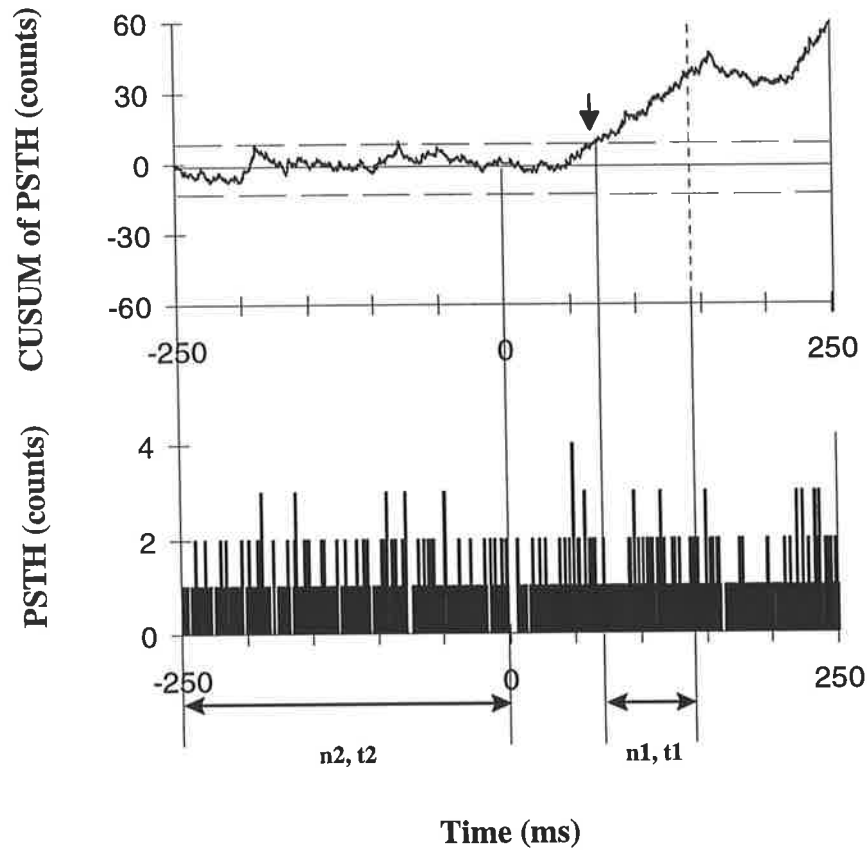
The subject was asked to perform a slow isometric ramp contraction of 40 N in 15 seconds to determine the force recruitment threshold of single motor units identified in an experimental session. Force recruitment threshold was determined as the bite force at which a single motor unit started to fire regularly. BF= bite force. The arrows indicate the force recruitment threshold.

#### 5.2.4 Data analyses

##### *Single motor units analyses*

During off-line analysis, the shape of the SMU action potentials was recognised using a template-matching algorithm (SPS-8701) that sent out recognition pulses whenever it matched the shape of a SMU action potential. The representation of a SMU in the Macro EMG (MacroRep, Stålberg, 1983) and the force recruitment threshold of a SMU were used to determine the size of the SMU. Two precautions were taken to make sure that at least two different SMUs were recorded simultaneously. Firstly, the shape of the MacroRep (Scutter & Türker, 1998) was obtained on-line and used to discriminate units. Secondly, the output of one of the SMU electrodes was used to trigger an oscilloscope where both SMU electrode outputs were displayed.

The reflex response of each motor unit was analysed by building a peristimulus time histogram (PSTH) of the SMU firing around the time of the stimulus. The CUSUM of the PSTH was constructed to determine the onset latency, duration and amplitude of the reflex responses (Ellaway, 1978; Buller *et al.*, 1980). The largest CUSUM deflection in the prestimulus period was obtained and this value was then used to make symmetrical 'error lines' (two horizontal dashed lines in Figure 5.3), which was similar to the 'error box' in Chapter 3.



**Figure 5.3 PSTH of a single motor unit**

CUSUM of PSTH (top) and PSTH (bottom) of a single motor unit are illustrated. In CUSUM, the maximum prestimulus deflection (error lines) are indicated by the horizontal dashed lines. The vertical dotted lines indicate the reaction time of the subject to this stimulus (140 ms). Any deviation in the poststimulus period, which exceeded the limits of the error lines and occurred before the reaction time, was considered a reflex response. An arrow indicates the latency of the excitatory reflex response in this unit. The stimulus was delivered at time zero. To determine the size of the reflex response, PSTH counts for the total duration of the reflex response were compared with the counts in the prestimulus period (-250 to 0 ms).  $n_1$  = counts in reflex response period,  $n_2$  = counts in prestimulus period,  $t_1$  = time period for reflex response,  $t_2$  = time period for prestimulus level.



For a poststimulus CUSUM deflection to be identified as a reflex response, it had to be larger than the error lines and had to occur before the reaction time to this stimulus (140 ms, Brodin *et al.*, 1993a). The time of onset of excitatory (up-going phase) or inhibitory (downward-going phase) responses were identified from the time when the poststimulus CUSUM crossed the limits of the error lines. The significance of a reflex response was tested by comparing the PSTH counts underlying the reflex response to the counts in the prestimulus period using a method described by Garnett and Stephens (1980). Thus, if  $n_1$  and  $n_2$  represent the counts observed in time periods  $t_1$  and  $t_2$ , the significance of the difference between the mean counts in the two periods was determined using the formula:

$$z = \left( \frac{n_1}{t_1} - \frac{n_2}{t_2} \right) / \sqrt{\left( \frac{n_1}{t_1^2} + \frac{n_2}{t_2^2} \right)}$$

For example, in Figure 5.3:

$$z = \left( \frac{43}{22.25} - \frac{342}{250} \right) / \sqrt{\left( \frac{43}{22.25^2} + \frac{342}{250^2} \right)} = 1.86$$

Using this statistic, the level of significance for the difference found between the frequency of counts obtained during the prestimulus and reflex response periods was  $P = 0.031$ . These calculations confirmed that all the reflex responses, determined from the CUSUM of the PSTH records using the error lines, reached significance of at least 5 % level. A  $P$ -value of less than 0.05 was considered statistically significant for all tests used in this study.

The averaged bin counts in the prestimulus and reflex periods were also used in a calculation which gave the percentage change in the bin counts during the reflex response compared with the prestimulus background level. This calculation was used to estimate the relative size of the reflex response. For example, in Figure 5.3, the size of the excitatory reflex response was 40% for this SMUs.

### *Size determination of single motor units*

In order to determine the size of SMUs recorded simultaneously in an experimental session, the force recruitment thresholds of SMUs were measured and expressed in Newtons. However, as there are limitations when using force recruitment threshold to indicate the size of SMUs (see Discussion for details), the amplitude of the MacroRep was also measured in the current study. Using the spike triggered averaging (STA) technique, the SMU was used to trigger the averaging process and the Macro EMG was used as the source (Stålberg, 1983). The MacroRep was obtained for each SMU and the amplitude recorded.

## **5.3 RESULTS**

### *5.3.1 Reflex Responses of Single Motor Units*

The reflex responses of 65 SMUs were recorded in 29 experimental sessions on 7 subjects. The reflex responses recorded in SEMG and bite force are listed in Table 5.1.

**Table 5.1 Reflex responses of SEMG and bite force to slow-rising tooth stimulus**

	SER	SIR	IER	No reflex	Total
<b>SEMG</b>	15	6	6	2	29
<b>Bite Force</b>	0	0	29	0	29

The reflex response of SEMG and bite force were recorded in 29 experimental sessions. SER= sole excitatory reflex responses; SIR= sole inhibitory reflex responses; IER= an inhibitory reflex response followed by an excitatory reflex response.

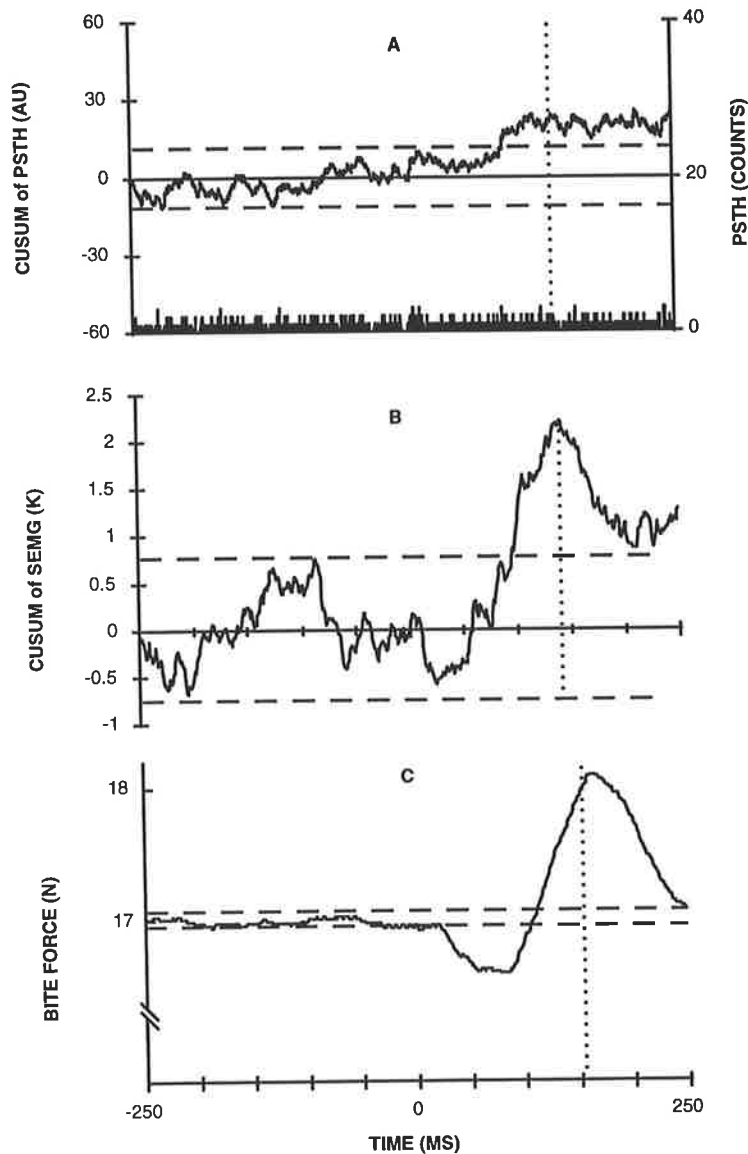
In 18 of these 29 experimental sessions, there was at least one SMU with a significant reflex response. The analysis was carried out on these 18 experimental sessions recorded from 6 subjects. Forty SMUs were recorded in these 18 experimental sessions. Of these 40 units, 26 showed a significant sole excitatory reflex response, 8 showed a sole inhibitory reflex response and 6 unit showed No reflex (Table 5.2).

**Table 5.2 Reflex response of single motor units**

	SER	SIR	IER	No reflex	Total
<b>SMU</b>	26	8	0	6	40

SER= sole excitatory reflex responses; SIR= sole inhibitory reflex responses; IER= an inhibitory reflex response followed by an excitatory reflex response.

Figure 5.4 demonstrates the reflex response of a single motor unit to a 2.5 N slowly-rising stimulus of the upper left lateral incisor (CUSUM of PSTH, CUSUM of SEMG and bite force).



**Figure 5.4** Reflex responses of a single motor unit to slowly-rising tooth stimulation

Changes of PSTH and its CUSUM (A), CUSUM of SEMG (B), and bite force (C) to 2.5 N push stimulation of upper lateral incisor are illustrated. In all recordings, the prestimulus control levels are indicated by horizontal dashed lines and vertical dotted lines demonstrated the reaction time. In the poststimulus period (0 - 250 ms), any deflection which was above the prestimulus control level and happened before the reaction time was considered as a reflex response.

From the CUSUM of PSTH recordings, the time of onset of the excitatory (up-going) phase or inhibitory (down-going) phase were clearly identified by the points when the deflections crossed over the prestimulus control level (error lines). The statistical significance of fluctuations in response change of motor unit firing was assessed (see 5.2 Methods for details). Therefore, this would be treated as no reflex response if the poststimulus deflection did not cross over the prestimulus control level or if the  $z$  value calculation did not reach the significance level.

### ***5.3.2 Reflex Responses of Different-Sized Single Motor Units***

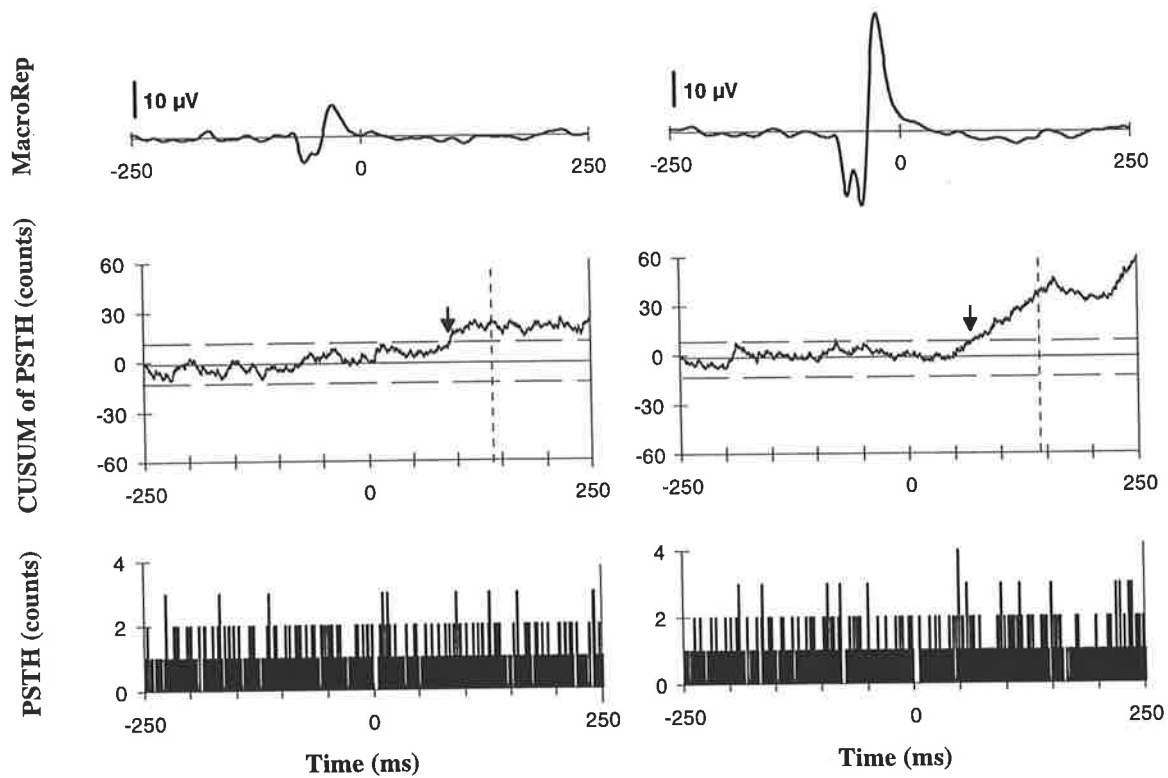
Within each of the 18 experimental sessions, two to three SMUs were identified and their reflex responses were recorded simultaneously. Two SMUs (a pair of SMUs) were recorded in 14 experimental sessions and three SMUs recorded in 4 experimental sessions (Table 5.3).

Figure 5.5 demonstrates the excitatory reflex responses of a pair of SMUs, which show that larger SMUs displayed larger excitatory and/or smaller inhibitory reflex responses than smaller SMUs.

**Table 5.3 Reflex responses of different-sized single motor units**

session	unit	macro	reflex	%reflex	threshold	frequency
1	1	415	SER	8	3	16
	2	1218	SER	34	8	25
2	3	708	SER	14	10	22
	4	825	SER	54	9	22
3	5	288	SER	48	6	18
	6	788	SER	64	8	13
4	7	232	SER	25	2	17
	8	1437	SER	60	4	13
5	9	376	0	0	4	17
	10	1623	SER	53	5	14
6	11	805	SER	32	10	13
	12	1709	SER	40	16	9
7	13	388	SER	78	3	16
	14	910	SER	35	10	16
8	15	376	SIR	-33	4	16
	16	908	0	0	15	15
9	17	498	SIR	-63	8	15
	18	961	SER	54	16	13
10	19	76	SER	9	5	21
	20	246	SER	55	14	14
11	21	205	SIR	-49	23	24
	22	229	SER	50	13	24
	23	266	0	0	26	15
12	24	88	SER	16	1	18
	25	178	SER	98	6	12
13	26	93	SIR	-55	2	19
	27	166	SER	27	6	13
14	28	68	SER	33	2	20
	29	107	SER	63	32	14
15	30	468	0	0	32	13
	31	1269	SER	98	20	11
	32	1732	SER	42	25	14
16	33	51	SIR	-32	30	14
	34	378	SIR	-73	11	14
17	35	190	0	0	40	14
	36	1039	SIR	-67	24	13
	37	1303	SER	30	48	13
18	38	332	SIR	-44	10	20
	39	1220	SER	71	10	13
	40	1362	0	0	10	12

The activities of 40 SMUs in 18 experimental sessions are illustrated. The MacroRep amplitudes of the SMUs are expressed as  $\mu\text{V}$ . The percentage change (%reflex) of SMU activity was calculated by comparing the reflex response with the background activity level of prestimulus period, minus values represent decrease of muscle activity. The firing frequency of SMUs are demonstrated in Hz. SER= sole excitatory reflex; SIR= sole inhibitory reflex, 0= no significant reflex response.



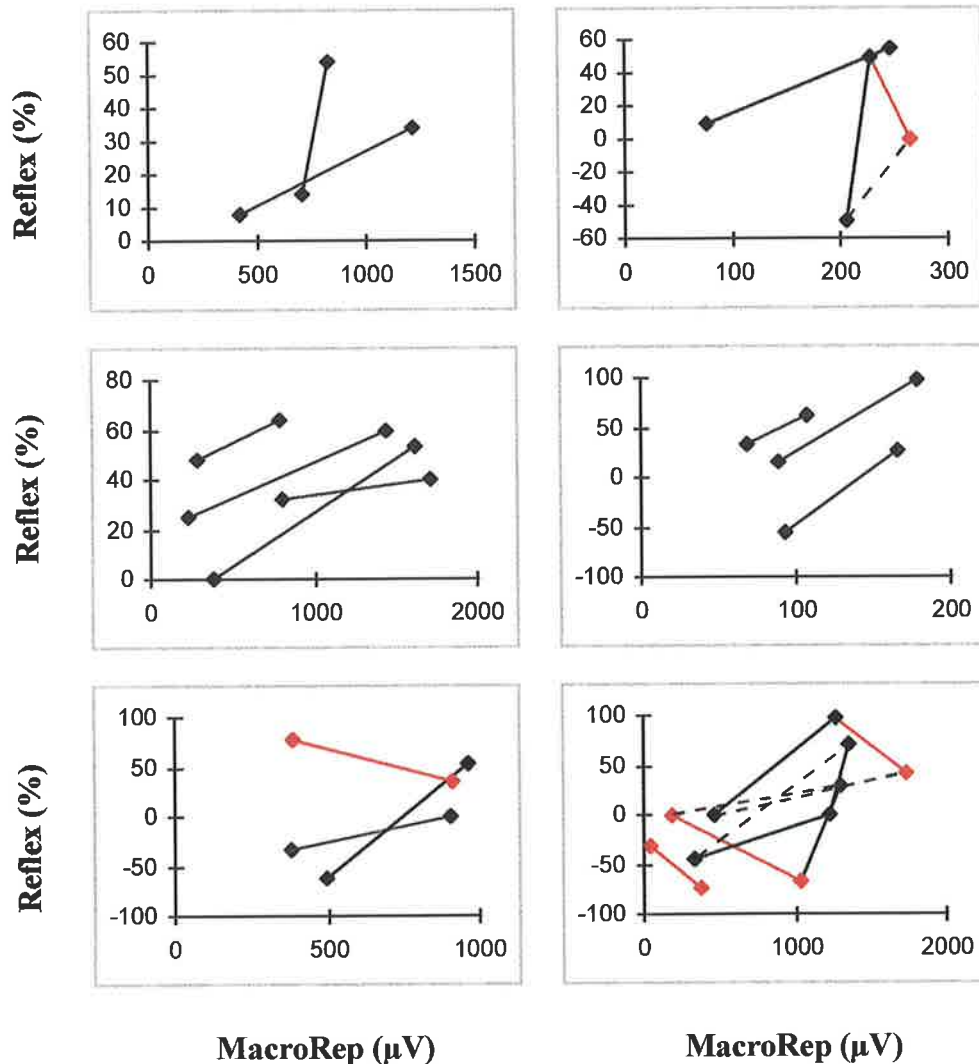
**Figure 5.5 Reflex responses of a pair of different-sized single motor units to slowly-rising tooth stimulation**

PSTH (bottom), CUSUM (middle) and MacroRep (top). In CUSUM, the maximum prestimulus deflection (error lines) are indicated by the horizontal dashed lines. The vertical dotted lines indicate the reaction time of the subject to this stimulus (140 ms). Any deviation in the poststimulus period, which exceeded the limits of the error lines and occurred before the reaction time, was considered a reflex response. The arrows indicate the latencies of the excitatory reflex responses in these units. The stimulus was delivered at time zero. To determine the size of the reflex response, PSTH counts for the total duration of the reflex response were compared with the counts in the prestimulus period (-250 to 0 ms). There was more excitatory reflex response in large-sized SMU than that of small-sized SMU.

The relationship between the reflex response and the MacroRep size of SMUs in 6 subjects was plotted (Figure 5.6). There were 21 pairs of SMUs in which larger-sized SMUs had larger excitatory or smaller inhibitory reflex response than those of small-sized SMUs; however, there were 5 pairs of SMUs showing the opposite (sign test,  $P < 0.05$ , Pagano, 1994b).

To express all the results in terms of supporting or opposing the orderly recruitment of SMUs, a rank score was calculated according to Kendall's method (Henneman *et al.*, 1965b) and tied ranks were assigned half score. If there were stronger excitatory or less inhibitory reflex in the small-sized SMU, the recruitment of the pair is referred to as the 'usual' order. The opposite of this is the 'reverse' order. The probability of random distribution illustrated that the reverse order was significantly more prevalent in these pairs ( $\chi^2 = 19.69$ ,  $P < 0.05$ ).





**Figure 5.6 Relationship between reflex response and MacroRep amplitude**

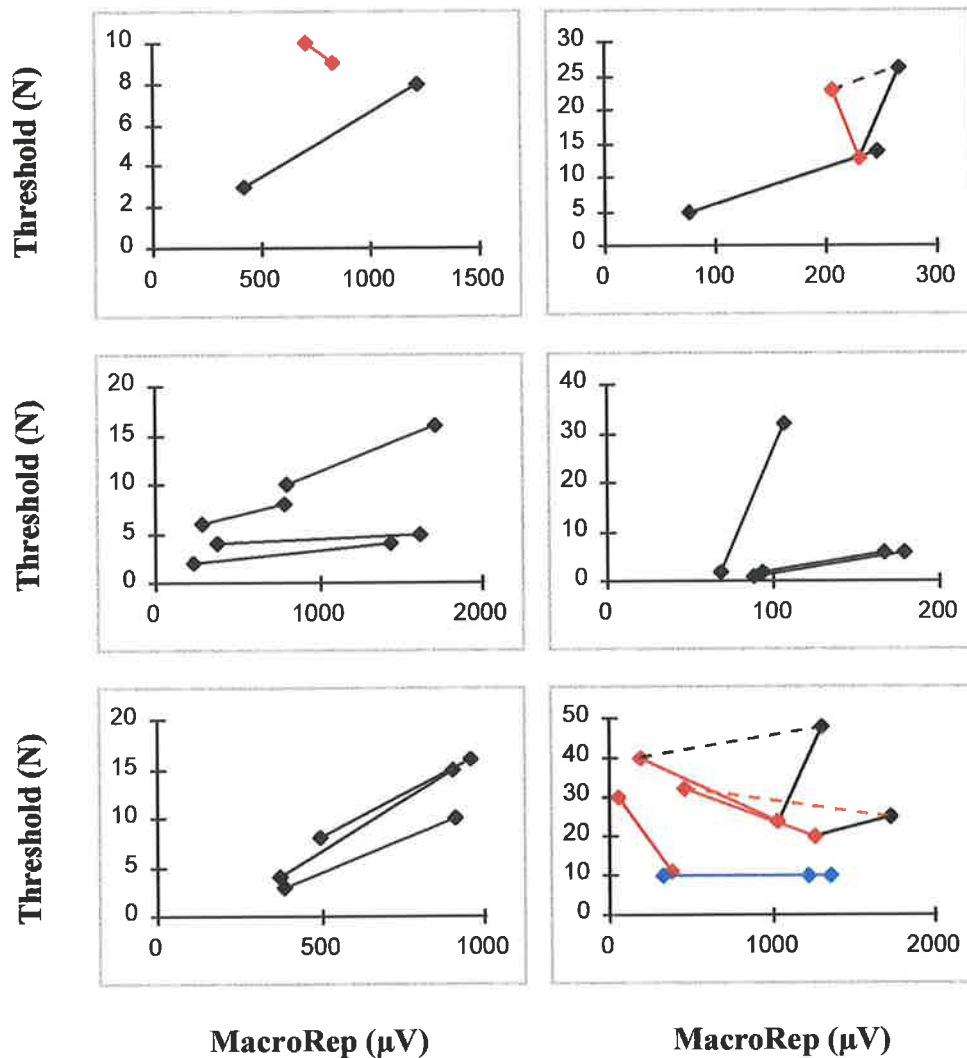
The size of the reflex response and MacroRep size of 40 SMUs, recorded in 18 experimental sessions from 6 subjects are illustrated. Black lines indicate that the larger-sized SMUs had larger excitatory or smaller inhibitory reflex responses than those of smaller-sized SMUs. Red lines indicate that the larger-sized SMUs had smaller excitatory or larger inhibitory reflex responses than those of smaller-sized SMUs. There were 3 SMUs (small, medium, and large SMUs) recorded simultaneously in 4 sessions and the relationships between small and large SMUs were illustrated by dashed line.

### 5.3.3 Firing Frequency of Different-Sized Single Motor Units

The discharge frequency of the 40 SMUs that constituted the 18 experimental sessions were compared using the paired t-test. There was significant difference ( $P < 0.05$ ) in the firing frequency of large and small motor units, which indicates that the small SMUs fired faster than large SMUs.

### 5.3.4 Force Recruitment Threshold of Single Motor Units

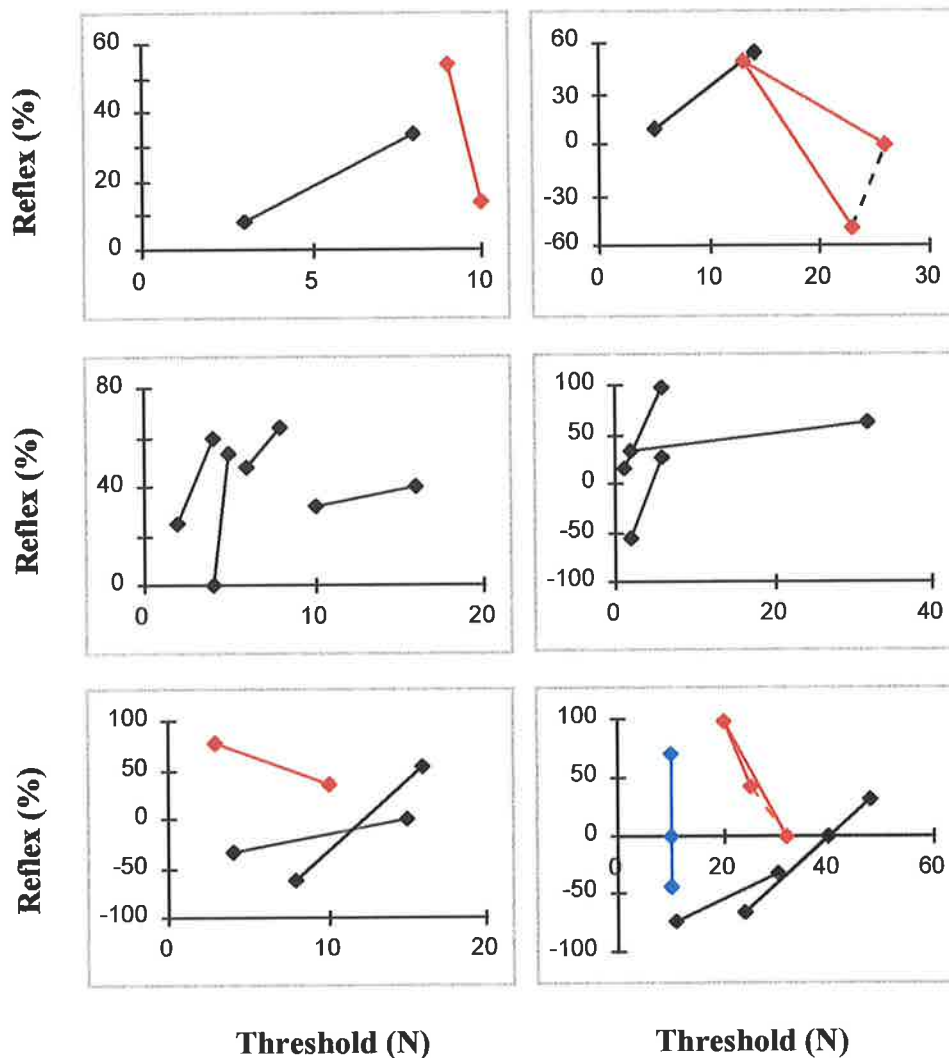
As described in Methods, the subject was asked to perform a slow isometric force ramp contraction of 40 N in 15 seconds to determine the force recruitment threshold of the motor units after each experimental session was completed. The force recruitment threshold of a SMU was determined as the lowest jaw closing force (the average value obtained from 3 attempts) at which the motor unit started to fire regularly. The relationship between the MacroRep and force recruitment threshold is shown in Figure 5.7, in which 17 pairs of SMUs showed a positive relationship and 6 showed a negative relationship between the size of MacroRep and the force recruitment threshold (sign test,  $P > 0.05$ ). On one occasion, all the 3 simultaneously recorded SMUs fired at 10 N and they were discarded in sign test. Using Kendall's method (Henneman *et al.*, 1965b), the probability of random distribution illustrated that the large-sized SMUs had significantly higher force recruitment threshold than the small-sized SMUs ( $\chi^2 = 9.31$ ,  $P < 0.05$ ).



**Figure 5.7 Force recruitment threshold and MacroRep amplitude**

Force recruitment threshold and Macro representative (MacroRep) amplitude of SMUs, recorded from 6 subjects are illustrated. Black lines indicate that the larger-sized SMUs had larger force recruitment thresholds than that of smaller-sized SMUs. Red lines indicate that the larger-sized SMUs had smaller force recruitment thresholds than that of smaller-sized SMUs. There were 3 SMUs (small, medium, and large SMUs) recorded simultaneously in 4 sessions. The relationships between small and large SMUs are illustrated by a dashed line. In one session, simultaneously recorded 3 units recruited at the same force level which are indicated by a blue line.

The relationship between the reflex response and force recruitment threshold is illustrated in Figure 5.8, showing that 16 pairs of SMUs had a positive relationship compared to 7 with a negative relationship (sign test,  $P > 0.05$ ). On one occasion, all the 3 simultaneously recorded SMUs fired at 10 N and they were discarded in sign test. Using Kendall's method (Henneman *et al.*, 1965b), the probability of random distribution illustrated that high threshold SMUs had more excitatory and less inhibitory reflex responses than those of low threshold SMUs ( $\chi^2 = 6.23$ ,  $P < 0.05$ ).



**Figure 5.8 Reflex responses and force recruitment thresholds of single motor units**

Reflex responses and force recruitment thresholds of SMUs, recorded from 6 subjects are illustrated. Black lines indicate that the high threshold SMUs had larger excitatory or smaller inhibitory reflex responses than those of low threshold SMUs. Red lines indicate that the high threshold SMUs had smaller excitatory or larger inhibitory reflex responses than those of low threshold SMUs. There were 3 SMUs (small, medium, and large SMUs) recorded simultaneously in 4 sessions. The relationships between small and large SMUs are illustrated by dashed line. In one session, simultaneously recorded 3 units recruited at the same force level which are indicated by a blue line.

## 5.4 DISCUSSION

### ***5.4.1 Distribution of Synaptic Inputs from Periodontal Mechanoreceptors to the Motoneuron Pool of Human Masseter***

In this study, the reflex responses of different-sized SMUs to periodontal mechanoreceptor stimulation using slowly-rising stimulus profiles are presented. The results show that the large SMUs receive more excitatory and less inhibitory inputs than those of small SMUs. Therefore, the application of periodontal input may reverse the recruitment order of motoneurons in tonic biting tasks where periodontal mechanoreceptors are activated continuously.

In most voluntary and reflex contractions studied to date, motor units are recruited into activity in an orderly fashion, ie. the size principle (Henneman *et al.*, 1965b). The neural mechanisms underlying orderly motoneuron recruitment have been the subject of considerable debate. Henneman's original explanation for the size principle was that the normal sequence of motoneuron recruitment is primarily determined by the intrinsic properties of the motoneurons themselves. For example, a smaller-sized motor unit has larger input impedance, therefore, a similar amount of excitatory current generates larger synaptic potential in smaller cells than it does in larger cells (Henneman *et al.*, 1965a,b). However, the properties and distributions of synaptic input have been shown to be equally important for determining the recruitment order (Henneman & Mendell, 1981; Burke, 1981; Gustafsson & Pinter, 1985; Pinter, 1990).

A variety of inputs to motoneuron pools have been used to examine whether the distribution of an input supports the size principle, ie. larger excitatory or inhibitory potentials in smaller motoneurons compared with the larger ones (Cope & Pinter, 1995). The results of these experiments in animals were usually in agreement with the expectations of the size principle (Henneman, 1957; Henneman & Olson, 1965; Henneman *et al.*, 1965a,b; Clamann *et al.*, 1974a,b). However, in all of these experiments, the distribution of the synaptic inputs were determined by recording the postsynaptic potentials. Since the size of a postsynaptic potential depends upon the input impedance of the cell, it does not necessarily indicate the magnitude of the input current received by the cell.

A better method is to record the actual synaptic current received by the cell. For example, Heckman and Binder (1990) found that the Ia effective synaptic current was not uniformly distributed to motoneurons of the medial gastrocnemius (MG) muscle in cat. The high input impedance motoneurons (small motoneurons) tended to receive about twice as much Ia effective synaptic current as the low input impedance motoneurons (large motoneurons). Therefore, the Ia input acts to greatly expand the range of recruitment thresholds generated by differences in the intrinsic properties of motoneurons. This would maximise the usage of fatigue-resistant units, and this lower input-output gain would be advantageous in movements requiring a high degree of precision - a precisely controlled movement with low force steps and slow speed.

Cutaneous afferent input is also not distributed equally to motoneurons. For some cells its effects are excitatory, while for others it is inhibitory (Garnett & Stephens, 1980).

Considering only the excitatory effects, the pattern for cutaneous input is roughly the reverse of that found for the group Ia input (Heckman & Binder, 1990). Kanda and Desmedt (1983) showed that in the decerebrate cat, stimulation of cutaneous afferents reversed the order of recruitment of motor units produced by muscle stretch. In human first dorsal interosseous (FDI) studies, cutaneous stimulation caused an increase in the recruitment threshold of units normally recruited at contraction strengths below 1.5 N and a decrease in the recruitment threshold of units recruited at contraction strength above 1.5 N (Garnett & Stephens, 1980). With the aid of cutaneous stimulation, therefore, the subject could recruit the unit which was previously recruited at a higher contraction level (Kanda *et al.*, 1977; Garnett & Stephens, 1980, 1981; Kanda & Desmedt, 1983).

The results of the present study are consistent with the cutaneous studies (Garnett & Stephens, 1980), that the large motor units have more excitatory and less inhibitory reflex responses than those of small motor units. This findings indicates that the distribution of the periodontal inputs is not equal but distributed differentially, favouring the larger motoneurons.

#### ***5.4.2 The Effect of Discharge Frequency***

It is well known that the discharge frequency of SMU can affect the amplitude of inhibitory reflex responses (Miles & Türker, 1986; Bonte & van Steenberghe, 1989; Miles *et al.*, 1989). Yamamura and his co-workers have demonstrated that the effects of reflex responses originating from the periodontal mechanoreceptors varied depending on the level of prestimulus firing frequency (Yamamura *et al.*, 1993;



Yamamura & Shimada, 1993). In the present study, it has been found that the smaller SMUs fired significantly faster than the larger SMUs when the activities of these units were recorded simultaneously (paired t-test,  $P < 0.05$ ). According to the frequency principle (Miles & Türker, 1986), the firing frequency would only affect the inhibitory reflex response (Miles *et al.*, 1989), in which the low firing frequency units (larger-sized SMUs) tended to have more inhibitory reflex response than the high firing frequency units (smaller-sized SMUs). However, it was found in this study that the larger-sized SMUs had more excitatory or less inhibitory reflex responses than those of smaller-sized SMUs. Therefore, differences in firing frequencies were unlikely to be responsible for the findings of this study. The present finding is not consistent with the findings of Yamamura and Shimada (1993) that when the different-sized motor units were tested at the same level of firing frequency, the small motor units tended to exhibit excitatory reflexes and the large motor units had inhibitory ones (Yamamura *et al.*, 1993; Yamamura & Shimada, 1993). Methodological differences may explain the differences in results. For example, using the amplitude and shape of SMU spikes, or using the force recruitment threshold of SMUs to determine the size of SMUs may not be appropriate (see discussion below). Furthermore, in that study the reflex response was assumed to continue for one second after the application of the stimulus (Yamamura *et al.*, 1993; Yamamura & Shimada, 1993). This is more than 7 times the reaction time that was measured in response to a similar stimulus to a tooth (Brodin *et al.*, 1993a).

### 5.4.3 Methods for Determining the Size of Single Motor Units

When Henneman first studied the phenomenon of orderly recruitment within a motoneuron pool, the size of the motor unit was determined from the amplitude of the nerve spike potentials of ventral root filaments (Henneman, 1957; Henneman *et al.*, 1965b). Since then, several researchers have confirmed orderly recruitment in intact animals (Clark *et al.*, 1978) and in human subjects (Goldberg & Derfler, 1977; Desmedt & Godaux, 1979) using the amplitude of spike potentials recorded in different muscles. This is an inappropriate indicator of motor unit size, since the spike component of the motor unit potential recorded with a selective electrode is generated by a small proportion of the muscle fibres in a motor unit (Stålberg, 1980).

In human studies, the force recruitment threshold of a SMU is used as the indicator of the size of the motoneuron, as this has been found to be the best indicator of the cell size in animals (Heckman & Binder, 1990). However, it has been recently shown that repeated measurements of the force recruitment threshold of a SMU in the jaw muscles is very variable, due to the changing contribution of the agonist and antagonist muscles to the total force (Scutter & Türker, 1998). Therefore, the absolute force recruitment threshold of a SMU can change considerably, depending on the participation of that muscle in the force ramp. In the present study, correlating the MacroRep and reflex findings to force recruitment threshold therefore gave weaker relationships (Figure 5.7 and 5.8) than the MacroRep against the reflex responses (Figure 5.6).

Positive correlations have been found between the force recruitment threshold and the peak twitch tension of SMU (Milner-Brown *et al.*, 1973c; Yemm, 1976,1977; Goldberg & Derfler, 1977). The twitch tension technique is most useful at low force levels where the motor units fire asynchronously and slowly. However, the use of the twitch tension has problems, especially when this technique is applied to the jaw muscles. A force transducer located between the teeth will underestimate twitch tension, due to the leverage system of the jaw (McMillan *et al.*, 1990). Furthermore, the muscle length, shortening velocity, co-contraction and motor unit firing rate, may all change the mechanical response of the motor unit (Hannam & McMillan, 1994).

This means that if the above criteria are used, the information obtained regarding the size of the SMUs can be misleading (Ertas *et al.*, 1995). More reliable information about the motor unit size may be obtained with SEMG, where all SMUs contribute to the recorded signal (Stålberg, 1980). For that, SEMG must be averaged by the discharge of a SMU to obtain the surface representation of the SMU (SurfaceRep). For example, (Eriksson *et al.*, 1984; Jones *et al.*, 1994) found that the unit with smaller SurfaceRep was recruited before the larger unit (Eriksson *et al.*, 1984). However, the distance of the SMU to the electrode on the surface varies and the amplitude of the surface representation of a SMU is significantly dependent on the location of the motor unit within the muscle (De Luca, 1979).

The best available technique for determining the size of human SMUs seems to be the representation of a SMU in the Macro EMG (Stålberg, 1980; Scutter & Türker, 1998). Since the representation of the motor unit in the Macro EMG (MacroRep) is a good

indicator of muscle unit cross sectional area, Stålberg and his colleagues (1986) have established that, in simultaneously discharging units recorded from a small area of a muscle, the unit with the smaller MacroRep comes from a smaller unit, and the larger MacroRep comes from a larger unit (Stålberg *et al.*, 1986). The MacroRep of SMUs have shown a very strong correlation with the recruitment properties of the SMUs (Scutter & Türker, 1998). Therefore, in the present study, the size of the SMU was estimated from the peak-to-peak amplitude of MacroRep.

In summary, the distribution of periodontal mechanoreceptors is not equal in the motoneuron pool of human masseter and the excitatory input distributes preferentially to the larger SMUs. These findings may suggest that, during normal chewing, the excitatory periodontal mechanoreceptor input may help the jaw closing muscles develop powerful forces to overcome food resistance as it is encountered (Abbink *et al.*, 1998). Therefore, less descending drive may be required to produce strong, active bite forces. It must be borne in mind that in the present study, the result is achieved when subjects were biting on less than 10% MVC which activates about 50% of SMUs in the motoneuron pool of masseter (Scutter & Türker, 1998). Further study is needed to determine the effect of periodontal mechanoreceptor input on higher recruitment threshold motoneurons.

## **CHAPTER 6**

### **CONCLUDING REMARKS**

This thesis has been concerned with the influence of periodontal mechanoreceptors on the reflex control of human jaw muscles. The reflex response of jaw muscles was induced by the mechanical stimulation of the periodontal mechanoreceptors. Slowly-rising push and rapidly-rising tap forces were applied to the teeth for this purpose.

Main conclusions from this study are:

1. The shape of the stimulus profile, the location of the stimulating probe and the presence of preload are the main factors that determine the reflex response of jaw closing muscles to mechanical stimulation of a tooth.
2. The reflex response of the masseter muscle is not a good representative of the net reflex response of jaw muscles. The reflex response of jaw muscles is best expressed by the averaged bite force. Using the averaged bite force, a net increase in bite force was dominant with the slowly-rising force application, whereas the rapidly-rising forces produced net reduction in the bite force.
3. In the human masseter, stimulation of the periodontal mechanoreceptors generates relatively large excitatory and less inhibitory reflex response in larger SMUs than those of smaller size. This finding implies that the inputs from the periodontal mechanoreceptors may distribute differentially to the masseteric motoneurons.

From the current studies, it appears that two relatively discrete reflex phenomena may occur in the response to periodontal stimulation. One is the inhibitory response that is prominent when the mechanical stimulus is applied rapidly. The other is an excitatory response that is prominent when the rate of application of the stimulus is slow. Whether one or both of these responses are evoked depends on the speed of the mechanical stimulus applied to the tooth. This stimulus rate dependency of the reflex may be the result of preferential activation of either low- or high-rate sensitive mechanoreceptors. The rapidly-rising force profiles may preferentially activate the high-rate sensitive, rapidly adapting receptors. The slowly-rising force profiles may preferentially activate the low-rate sensitive, slowly adapting receptors.

### **Periodontal mechanoreceptors can provide positive force feedback as well as protective functions**

The importance of sensory feedback from periodontal mechanoreceptors during mastication has been addressed by different groups of researchers. It is a well established view that periodontal mechanoreceptors can cause an inhibition in the jaw closing muscles. Clearly, a major function of this reflex is to reduce damage and protect teeth and the soft tissue when a hard object is suddenly encountered. Furthermore, it has been argued that the periodontal mechanoreceptors which cause inhibition of jaw closing muscles do not contribute to the excitation of the jaw closing muscles. Knowledge of such an excitatory mechanisms is important because it could represent a mechanism whereby bite force could be reflexly increased upon tooth-food-tooth contact.

From the observation in anaesthetised animals where mastication was induced by cortical stimulation, it has been shown that periodontal mechanoreceptors provide strong positive feedback to the jaw closing muscles. The present studies establish that periodontal mechanoreceptors in man also have the capacity to contribute to the control of jaw muscle activity. It has been clearly demonstrated that periodontal mechanoreceptors can provide positive feedback to bite force if a slowly-rising force is applied. When the slowly-rising force is applied to the tooth, the excitatory reflex responses (increase of bite force) are dominant; while with the rapidly-rising force, the inhibitory reflex responses (decrease of bite force) are dominant. Therefore, the different responses observed in earlier studies can be explained by different methods of stimulation used in different experimental protocols, in particular, the stimulation can produce rapid or slow tooth displacement which may differentially activate different reflex pathway.

#### **Possible reflex pathway between periodontal mechanoreceptors and jaw muscles**

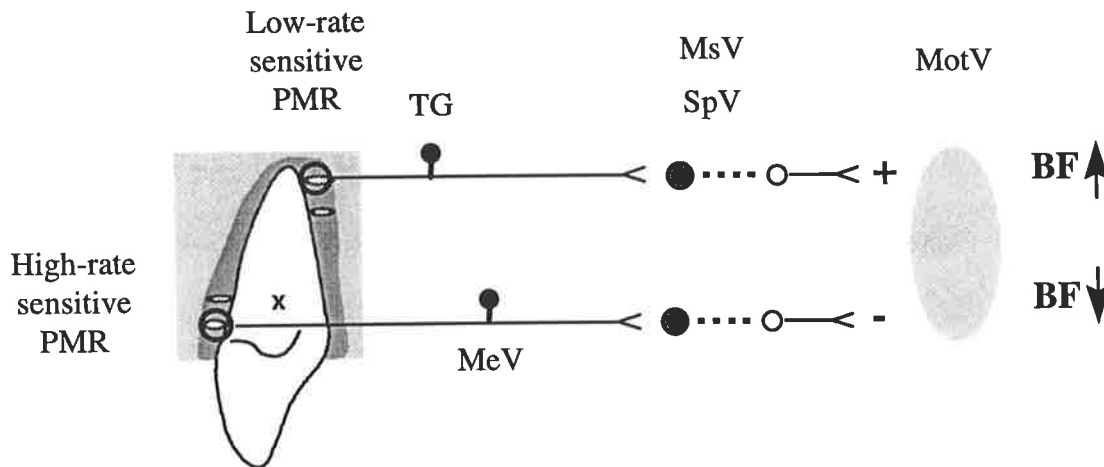
The central neural pathways of periodontal mechanoreceptor afferents are not so clear. There is much controversy and uncertainty surrounding the pathways of these afferents into the brain stem and to higher centres.

Most of the afferent nerve fibres arising from the periodontal ligaments terminate in the trigeminal sensory complex which contains the trigeminal main sensory nucleus and trigeminal spinal tract nucleus. It has been reported that a pathway for the

periodontal masseteric reflex induced by a tap to a tooth involved the trigeminal mesencephalic nucleus and the periodontal masseteric reflex induced by a constant pressure involved the trigeminal main sensory complex nucleus. Tap-induced response is dependent on the rate of rise of the initial phase of mechanical stimulation to the tooth, while constant pressure induced response is dependent on the intensity of the stimulation.

Considering this information and the findings in the present study, the reflex pathway between periodontal mechanoreceptor and jaw muscles is speculated (Figure 6.1). The slowly-rising push force may preferentially activate the low-rate sensitive, slowly-adapting mechanoreceptors that are more likely situated around the apex of tooth root and have their cell bodies in trigeminal ganglion. The afferent nerve fibres arising from these receptors terminate in the trigeminal sensory complex. From there, they may have excitatory connections with the motoneuron that innervate the jaw closing muscles. The latency of excitatory reflex indicates a polysynaptic pathway between periodontal mechanoreceptors and jaw closing muscles. However, so far, it has not been possible to identify the exact reflex pathway or to deduce the number of synapses in the pathway. It is possible that a substantial temporal summation is required to elicit excitatory reflex response. It is also possible that the polysynaptic pathway for this excitatory reflex traverses the motor cortex.





**Figure 6.1** A speculative wiring diagram of periodontal mechanoreceptors and jaw muscles

Low-rate sensitive, slowly adapting periodontal mechanoreceptors (PMRs) whose cell bodies are located in trigeminal ganglion (TG), may have an effect on the activity of the motor nucleus of the trigeminal nerve (MotV) causing a reflex increase of bite force (BF). On the other hand, high-rate sensitive, rapidly adapting PMRs whose cell bodies are situated in the trigeminal mesencephalic nucleus (MeV), may cause a reflex decrease of bite force. The cell bodies of PMRs and trigeminal motor nucleus are connected by interneurons in main sensory trigeminal nucleus (MsV) and spinal trigeminal tract nucleus (SpV). x= fulcrum.

The rapidly adapting periodontal mechanoreceptors would be activated by the rapidly-rising (tap) stimulus since they are very sensitive to rate change of the stimulation force. They have their cell bodies situated at the trigeminal mesencephalic nucleus. From there, via interneurons, they may induce an inhibitory reflex response of jaw closing muscles. The inhibition is thought to be mediated by an oligosynaptic probably disynaptic circuit.

These two feedback mechanisms demonstrated by the different patterns of reflex responses to slowly-rising and rapidly-rising tooth stimulation have clear functional significance. During chewing, the mechanoreceptors around the teeth are optimally placed to register the force being applied to the teeth. When the teeth begin to bite into food, the activation of periodontal receptors by the pressure exerted on the teeth can control the level of muscle activity. Reflexly induced facilitation may help the muscles to maintain pieces of food between the teeth so that they may then be crushed. However, when one bites unexpectedly on a rigid object, the resulting brisk forces on the teeth will evoke a strong afferent volley from the periodontal receptors, which reflexly inhibits the powerful jaw closing muscles to protect the teeth and their supporting tissues.

### **Excitatory inputs from periodontal mechanoreceptors distribute differentially to masseter motoneurons**

Some functional significance has previously been sought for the fact that most muscles are heterogeneous with regard to motor unit mechanical properties. In

particularly, it has been suggested that fast and slow contracting motor units might be involved selectively in different types of movement. The consequence of the size principle is that no specialised distribution of the input to a motoneuron pool is needed for the orderliness of recruitment. This means that every motoneuron shares qualitatively identical inputs, therefore, the motoneuron pool would seem to be organised in such a way as to prevent selective activation of different sub-populations of motor units.

The current study has shown that the larger SMUs are likely to receive more excitatory and less inhibitory reflex response than those of smaller size. This may cause recruitment of motoneurons contrary to the size principle under some circumstances. The potential seems to exist for selectively activating different populations of motor units during the reflex contraction of the masseter muscle.

Taking the present results together with the observations in man on the effects of cutaneous stimulation on recruitment of hand muscles, it seems that the order of motor unit recruitment is not in fact invariable, but can be influenced by afferent input. Here, perhaps lies the significance of these results in functional terms. The teeth come in contact with many different types of objects which give rise to the periodontal mechanoreceptor input of various types and intensities. Different types and intensities of the periodontal mechanoreceptor input could be distributed differentially to motoneurons of jaw muscles. The functional advantage of having separate access to different population of motor units may depend on the different needs of tasks. If the

excitatory input is more effective on the large motoneurons, as suggested by the findings of this study, it will be the large, strong and fast SMUs which are recruited preferentially when the teeth are stimulated by a constant or slowly-rising pressure. The differential recruitment of larger SMUs can provide the capacity to produce large bite forces with the minimum effort and help generate power stroke where resistance between the teeth is reduced. However, this feedback system is risky since large forces are developed reflexly. Any sign of a sudden increase in the resistance between the teeth would initiate inhibitory reflex which overwhelms the positive feedback. This works as a security mechanism to make the masticatory system function well.

### **Limitation of these studies**

It might be premature to extrapolate the results from the present study to the reflex control of jaw muscles during masticatory movements. In the present study, reflex responses were elicited in jaw muscles of humans when the jaws were fixed. During masticatory movement, the amplitude and the direction of the forces applied to the teeth will be different. For example, most of the forces applied to the incisor teeth are directed axially during mastication. However, in the current studies, horizontal forces were used. Although there will be significant vectors of this force in the labial-palatal direction, and the majority of periodontal afferents respond to forces applied both axially and horizontally, the ideal direction for stimulation force is vertical for simulating the direction of chewing force. Unfortunately, the experimental apparatus did not allow using this kind of force application.

During mastication the magnitude of bite forces generated are higher than those used in the present study. Forces up to 70 - 150 N have been recorded in humans during normal mastication (Anderson, 1956a,b; Anderson & Picton, 1958). When the force thresholds of the periodontal mechanoreceptors were considered (10 - 800 mN), it is very likely that the bite forces that occur during mastication will activate a large number of mechanoreceptors. Many of these receptors would not be situated in the periodontium but nevertheless they may affect the activity of jaw muscles. Therefore, the periodontal mechanoreceptors are unlikely to be the only group of sensory receptors to contribute to the reflex control of jaw muscles during mastication and probably form only a part of the total input. Muscle spindles in the jaw muscles and temporomandibular joint receptors are other possible candidates to provide an excitatory input to the jaw closing muscles.

Finally, during the power stroke phase of chewing where food is crushed, the chewing task is more likely to be performed by the molar teeth, rather than the incisor teeth. It has also been reported that the receptors in the molar region have different physiological characteristics from those found in the incisor regions. Since in the present studies, all reflex responses are elicited by stimulating the receptors in the incisor area, the interpretations of these studies should not be generalized to include other teeth or regions.

## CHAPTER 7

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**CHATER 8****APPENDIX****8.1 CURRICULUM VITAE****Personal**

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Marital Status:	Married
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Present Position:	Postgraduate student
	Department of Physiology
	The University of Adelaide
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**Academic Qualifications**

Bachelor of Dentistry:	Awarded in August, 1989, issued by Capital Institute of Medicine, Beijing, P.R. China.
Master of Dentistry:	Awarded in October, 1992, issued by Capital Institute of Medicine, Beijing, P.R. China.

**Scholarships and Awards**

1984 - 1989	First-class scholarship, awarded by the Capital Institute of Medicine, Beijing, P.R. China.
1995-date	Adelaide University Scholarship.

**Positions held**

1992 - 1993	General practitioner in Beijing Stomatological Hospital, Capital Institute of Medicine, P.R. China.
1993 -1995	Occupational Trainee in Orthodontic Unit, Department of Dentistry, The University of Adelaide.
1995 - date	Postgraduate student in Department of Physiology, The University of Adelaide.

**Publications**

1. Yang J, Jin YL (1994) Developmental changes in craniofacial width and asymmetry in North Chinese population. *Journal of Orthodontics* 1: 116-118.
2. Türker KS, Yang J, Brodin P (1997) Conditions for excitatory or inhibitory masseteric reflexes elicited by tooth pressure in man. *Archives oral Biology* 42: 121-128.
3. Scutter SD, Türker KS, Yang J (1997) A new method for eliciting and studying H-reflexes in the human masseter. *Archives oral Biology* 42: 371-376.

4. Türker KS, Yang J, Scutter SD (1997) Tendon tap induces a single long-lasting excitatory reflex in the motoneurons of human soleus muscle. *Experimental Brain Research* 115: 169-173.
5. Yang J, Türker KS (1998) Jaw reflex evoked by mechanical stimulation of teeth in man. *Journal of Neurophysiology* 81: 2156-2163.
6. Türker KS, Scutter SD, Yang J, Jenkins M, Tucker KJ, and Abolfathi PP (1999) Reflex control of masticatory muscles in man. *Neurobiology of Mastication-from Molecular to System Approach*. Edited by Nakamura Y and Sessle BJ. Elsevier Science, Amsterdam pp. 337-353.
7. Yang J, Türker KS (1999) Distribution of periodontal afferent input to motoneurons of human masseter. Submitted to *Archives oral Biology*.

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1. Yang J, Türker KS (1995) Sole excitatory periodontal-masseteric reflex. *Proceedings of The Australian Physiological and Pharmacological Society* 26:230P
2. Türker KS, Yang J, Scutter SD (1996) How silent is the 'silent period'. *Proceedings of the Australian Neuroscience Society* 7: 51
3. Türker KS, Jenkins M, Scutter SD, Yang J (1996) A method for quantifying reflex responses from the surface electromyogram. *Proceedings of the Australian Physiological and Pharmacological Society* 28: 37P
4. Yang J, Türker KS (1996) Periodontal mechanoreceptor input to the different-sized motor units in human masseter. *Proceedings of the Australian Physiological and Pharmacological Society* 28: 92P

5. Yang J, Türker KS (1997) Changes in the bite force in response to tooth pressure stimulation in man. *Proceedings of the Australian Neuroscience Society* 8: 175
6. Scutter SD, Türker KS, Yang J (1997) A new method for eliciting and studying H-reflexes in the human masseter. *Proceedings of the Australian Neuroscience Society* 8: 175

## **8.2 PUBLISHED PAPERS RESULTING FROM THIS THESIS**

Reprints of published papers associated with this thesis are shown overleaf

K.S. Türker, J. Yang and P. Brodin (1997) Conditions for excitatory or inhibitory masseteric reflexes elicited by tooth pressure in man.  
*Archives of Oral Biology*, v. 42 (2), pp. 121-128, February 1997

NOTE: This publication is included in the print copy of the thesis held in the University of Adelaide Library.

It is also available online to authorised users at:

[http://dx.doi.org/10.1016/S0003-9969\(96\)00112-4](http://dx.doi.org/10.1016/S0003-9969(96)00112-4)

Yang, J., and Türker, K.S., (1999) Jaw reflexes evoked by mechanical stimulation of teeth in humans.

*Journal of Neurophysiology*, v. 81 (5), pp. 2156-2163.

NOTE:

This publication is included in the print copy  
of the thesis held in the University of Adelaide Library.

Turker, K.S, Scutter, S.D., Yang, J., Jenkins, M., Tucker, K.J., and Abofathi, P.P. (1999) Reflex control of masticatory muscles in man. In: Neurobiology of Mastication: from molecular to systems approach. Edited by Nakamura, Y. and Sessle, B.J., Elsevier Science, Amsterdam pp. 337-353

NOTE: This publication is included in the print copy of the thesis held in the University of Adelaide Library.