STRETCH REFLEXES IN HUMAN MASSETER

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LIST OF ILLUSTRATIONS

ABSTRACT

The stretch reflex has been intensively studied in a number of spinal systems because of its importance as a manifestation of a major mechanism in the control of movements, posture, and locomotion, and because of its importance in clinical medicine. The aim of the present study was to investigate the pattern of reflexes evoked by stretch in a human jaw-closing muscle (masseter) at the level of the whole muscle and individual motor units. This matter is of particular interest because of earlier reports that the pattern of stretch-evoked responses in masseter differs from that of spinal muscles.

In order to study the reflex, a special-purpose stimulator was designed and built in this laboratory. This incorporated a number of unique features to match its performance to the special properties of the masticatory system. During my preliminary analysis of the surface electromyographic data, I developed a new analytical method with general application to signals of this type. This provides a quantitative index of muscle activity evaluation and is also free from the artefacts that conventional methods are known to produce in certain circumstances.

In contrast to earlier reports, the reflex response of the masseter to stretch was found to consist not only of a short-latency excitation, corresponding to monosynaptic projections of Ia afferents onto the homonymous motoneurons, but also of a long-latency phase which represents the output of a polysynaptic pathway. The later phase is more important physiologically, as it is this phase of excitation that produces active force in a reflex paradigm.

The study of the responses of individual motor units revealed further details of the organisation of this reflex. Among other things, some motoneurons were found to lack any physiologically significant projections from Ia afferents. Finally, I have developed a new method for estimating the shape of a compound post-synaptic potential evoked in a motoneuron by a sensory input.

DECLARATION

(statement of originality / consent to photocopy and borrow)

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AIMS AND GENERAL INTRODUCTION

The Stretch Reflex and Human Masticatory System

The stretch reflex was initially described by Liddell and Sherrington in the decerebrate cat in 1924. Since then it continues to be studied in animal preparations as well as in healthy humans and patients with neurological disorders of motor function. This continued interest is due to the fundamental role in motor control, in particular in maintaining posture, that was immediately assigned to the stretch reflex. This interest was supported by the possibility that a better understanding of the stretch reflex would find clinical applications. The tendon jerk has in fact become a routine procedure in every neurological examination and a tool providing valuable diagnostic information about the state of the patient's nervous system.

Since the initial studies of Liddell and Sherrington, a much better understanding of the stretch reflex and its role has been achieved and a certain evolution of the views on its mechanism and role has occurred. In particular, the emphasis has shifted from the short-latency phase of the reflex, determined largely by monosynaptic projections from the muscle spindles onto homonymous motoneurons, to the later phase of the reflex, which is the result of integration of all afferent information and its processing by the CNS at all levels up to the motor cortex. We now have more detailed information about afferent sources of the stretch reflex, the reflex pathways and neural structures involved, and the mechanical action of the stretch reflex (see Matthews, 1992). Comparison of the reflex responses of a number of skeletal muscles to stretch has provided us with the insight on the organisation of various motor systems, their structure determining their function.

The masticatory system, while similar in many respects to other motor systems, does have a number of unique features. They are determined by the gross anatomical features, such as teeth and tongue, and by its masticatory and speech functions, which are performed along with the postural function, common for many of the motor systems. The structure of the masticatory system has been also shown to be unique in many respects, this being true

for all of its elements: receptors and afferents, neural organisation and reflex pathways, efferents and muscles. Studying this system is essential for our understanding of these functions which are critical for survival of a living organism, and also provides basic knowledge in clinical areas such as neurology, oral surgery, dentistry, speech pathology etc. From the viewpoint of motor control studies, the masticatory system is interesting because it tests the generality of some of the concepts and ideas in the field that have been largely developed from studies of spinally innervated muscles.

The aim of this study was to study the stretch reflex in a jaw-closing muscle (masseter) of healthy humans. The reflex responses to slower stretches were a particular focus of this study, since there are few experimental data for the masticatory muscles. This, however, have been studied intensively in many limb muscles over the last thirty years, revealing the important role of the long-latency phase of the stretch reflex. This study presents a detailed investigation of the stretch reflex in human masseter muscle at the levels ranging from the overall muscle response to individual motoneurons. I also discuss a number of methodological questions and introduce novel quantitative methods of data analysis, which emerged during the course of this study.

CHAPTER 1

LITERATURE REVIEW

1.1 MASTICATORY SYSTEM

1.1.1 Muscles of Mastication

Masticatory muscles of man can be grouped into the jaw-closing muscles (*temporalis*, *masseter*, *medial pterygoid*) and the jaw-opening muscles (*digastric*, *lateral pterygoid* and the *suprahyoid* group). If the closing muscles are thought of as extensors and the opening muscles as flexors, then the basic reflexes observed would be similar to that observed in other motor systems, this division being, however, a simplification (Luschei & Goldberg, 1981). The morphology of masticatory muscles varies significantly between species, but generally is very complex (Rowlerson, 1990). Jaw-closing muscles are layered, multipennate muscles and the direction of pull can vary during closing, and may be different in adjacent layers. It is a common finding that masseter has three principal layers that are incompletely separated from each other. The temporalis muscle is also usually divided into two parts (anterior and posterior), or more.

In contrast to some limb muscles, jaw muscles do not have well-developed tendons. This, as well as difficult access to the motor nerves, makes them difficult to study by conventional physiological techniques. There have been no studies in humans in which electrical stimulation has been used to determine the mechanical properties of the jaw muscles. The motor unit contraction times were estimated in human masseter using the spike-triggered averaging (STA) technique. In the study by Yemm (1977) the masseter motor units had a continuous range of contraction times (25-90 ms). Goldberg & Derfler

(1977) and Nordstrom, Miles & Veale (1989) took into consideration the distortions of the twitch shape obtained by STA technique that may occur due to the firing pattern. The range of contraction times for masseter muscle was found to be 20-50 ms with two exceptional motor units with the slower contraction times about 65 ms (Nordstrom & Miles, 1990). There is also not much reliable information on fatigability of human masticatory muscles. From maximal biting force experiments there is evidence that the masseter is more resistant to fatigue by repeated maximal contractions than limb muscles (van Steenberghe, de Vries & Hollander, 1978; Clark & Carter, 1985). There are also no data on the physiological types of motor units of masticatory muscles in animals or humans compatible with the standardised test of Burke, Levine, Zajac, Tsairis & Engel (1971). An alternative method of STA has been used (Nordstrom & Miles, 1990), and in this study human masseter was found to be comprised predominantly of fast-twitch motor units with a broad spectrum of fatigability (corresponding to types FF, FR and FI). Very few physiological type S units were found, despite histochemical evidence for a substantial population of type I fibres in the masseter. In contrast to limb muscles, no correlation was found between twitch tension, contractile speed and fatigability (Yemm, 1977; Goldberg & Derfler, 1977; Nordstrom & Miles, 1990). This may be explained by the fact that masseter has very few physiological type S motor units. In limb muscles this correlation is strongly influenced by pooling of data from the different motor unit types. The analysis of data within a group of the same physiological type may weaken or eliminate this correlation.

The histochemical properties of masticatory muscles, on the contrary, have been studied extensively (Rowlerson, 1990). They were found to differ from histochemical properties of limb muscles in the following aspects:

1. generally smaller diameter of type I and II fibres than for the corresponding types in other muscles;

2. the diameter of type I fibres is larger than that of type II fibres, in contrast to the normal situation in other limb muscles;

3. masseter contains a larger proportion of fibres with intermediate staining for MATPase;

4. masseter muscle contains very few fibres that could be classified as type IIA;

5. masticatory muscles do not have the normal mosaic pattern of fibre type distribution, but rather have large groups of densely packed fibres of the same histochemical type.

In the extensive autopsy study by Eriksson & Thornell (1983), the overall proportions of the various fibre types in the masseter of 5 young male subject were: I, 62.5%; IIA 2.1%; IIB, 26.7%; IIC, 2.7% and IM, 6.0%. IIC and IM are intermediate staining types that are normally not found in significant numbers in limb muscles.

In summary, it appears that masticatory muscles, and masseter in particular, possess a combination of physiological, histochemical and anatomical properties that differ from the relationships widely accepted for the limb muscles.

1.1.2 Proprioceptors

Receptive organs which signal to the CNS information about the relative positions of the body parts are called proprioceptors. They should be distinguished from the other group of receptive organs, exteroceptors, which provide information about the position of the body in space. The receptors involved in proprioception lie in the muscle (muscle spindles and Golgi tendon organs), the joints and the skin. In the case of the masticatory system, periodontal mechanoreceptors and receptors of the oral cavity should be also considered as proprioceptors. The characteristics of these receptors and the role they play in the masticatory system are summarised below.

1.1.2.1 Muscle Receptors

Muscle spindles

The typical muscle spindle (Boyd, 1985) consists of a bundle of specialised muscle fibres (intrafusal fibres) which lie in parallel with the extrafusal fibres of the skeletal muscle. The intrafusal fibres are about 10 mm long, which is shorter than muscle fibres of the main muscle, and they may be attached to extrafusal fibres or tendinous insertions. There are two types of intrafusal fibres in the spindle: bag and chain fibres. On the basis of physiological properties, bag fibres have been subdivided into dynamic bag_1 and static bag_2 types.

The sensory innervation of the spindles is of two types: larger diameter Ia afferents distributing primary endings in the central area of the fibre and secondary group II afferents. Primary afferent endings terminate on all types of fibres, whereas there are few secondary endings in bag_1 fibres and they usually have terminals on bag_2 and chain fibres. The motor supply to the intrafusal fibres of the muscle spindle consists mainly of the small diameter γ neurones. In some cases the motor supply arises partially from branches of α-motoneurons innervating the extrafusal fibres. Such axons are known as β axons. Separate γ innervation of muscle spindles is found in mammals only. It has

been suggested that γ-innervation is a valuable evolutionary improvement allowing independent control of extrafusal and intrafusal motor systems. It should be mentioned that the terms "α", "β" and "γ" are not strictly applicable in the case of the jaw muscle nerves, because the conduction velocities are not the same as in the hindlimb and the diameter spectra have not been shown to have peaks clearly related to function (Taylor, 1990).

In normal physiological conditions muscle spindles have both velocity (dynamic) and position (static) sensitivity. The velocity sensitivity is more pronounced for primary endings. The firing pattern of secondary endings usually follows the displacement very well and the dynamic component in their firing patterns is small. The firing pattern of the afferents depends largely on the incoming γ-efferent activity. Primary endings respond in two different ways to stimulation of γ neurones. Stimulation of static fusimotor axons (γ_s) increases the resting level of discharge and the static component of the response to stretch. Stimulation of the dynamic fusimotor axons (γ_d) also increases the resting level of firing, but not as much as γ_s; its main effect is to produce a much greater dynamic response. An initial burst of firing has been also observed in primary endings at the onset of stretch (Brown, Goodwin & Matthews, 1969), which can be explained by the presence of cross-bridges between actin and myosin in the poles of the intrafusal bag fibres. These resist any extension with a high short-range stiffness up to a critical point and most of the stretch will therefore be taken by the central, poorly-striated part of the fibre. The sensitivity of primary endings to stretch is much greater for small rather then large stretches because of this. They are called non-linear receptors, exquisitely sensitive to small displacements (Matthews & Stein, 1969). The response of primary and secondary afferents has been shown to depend on the previous pattern of activation (Proske, Morgan & Gregory, 1992).

Reviews of muscle spindle distribution, properties and function in the masticatory muscles have been presented recently (see chapters by Rowlerson, Appenteng and Taylor, in Taylor (ed.), 1990). Muscle spindles have been found essentially only in jaw-closing

muscles, with the majority of them lying in the deep and anterior portions of the muscles. They have been found to contain typical bag_1, bag_2 and chain fibres (Rowlerson, Mascarello, Barker, & Saed, 1988), suggesting that they are broadly similar to those in other muscles. It has proved difficult in animal experiments to demonstrate two distinct groups of primary and secondary spindle afferents (Appenteng, 1990). Estimates based on measurements of afferent conduction velocities have shown unimodal distributions with the mean value about 50 $m.s^{-1}$, in contrast with bimodal distribution in skeletal muscles. The dynamic indices of spindle response to a stretch, estimated in the same experiments, have not shown any distinct groups of afferents also. However, it appears possible to identify afferents as primary and secondary on the basis of dynamic indices after suxamethonium infusion (Cody, Lee & Taylor, 1972; Inoue, Morimoto & Kawamura, 1981). The rationale for this test was Rack & Westbury's (1966) demonstration that the dynamic response was enhanced by suxamethonium infusion (see also Boyd, 1985). Inoue *et al.* (1981) have shown that masseter afferents have a remarkably high position sensitivity (7.5 $impulses.s^{-1}$ frequency increase per 1% of resting length increase, this being about 3 $impulses.s^{-1}$ per 1% of length increase in cat and human limb muscles). There is still a limited number of investigations of the properties of spindles in masticatory muscles, and many aspects of their function needs further investigation.

Golgi tendon organs

Golgi Tendon organs are contraction-sensitive mechanoreceptors of mammalian skeletal muscles innervated by fast-conducting Ib afferent fibres. These receptors have recently been comprehensively reviewed (Jami, 1992). They lie in the musculo-tendinous or musculo-aponeurosis junctions. They are composed of a spindle-shaped connective tissue capsules enclosing a number of collagen strands that are attached to the tendon at one end, and to about 10-20 individual muscle fibres at the other end. The afferent fibre is of large diameter (group Ib; 10-20 μm; 60-110 $m.s^{-1}$ in the cat, measured in the

vicinity of the spinal cord). There is a complete overlap of the conduction velocities of Ib and Ia (spindle) afferents.

Tendon organs have become accepted as very sensitive receptors of muscle force with a widespread role in movement control. Houk & Henneman (1967) found that a tendon organ could respond to stimulation of just one α-motor axon, innervating a single motor unit. It was shown also in the same experiment that stimulation of a small number of motor units could excite one previously silent tendon organ and silence another one, previously firing tonically. This effect is probably due to the unloading of the latter by in-parallel contraction of neighbouring motor units that do not insert a fibre into this tendon organ. However, usually a motor unit attaches only one fibre into the tendon organ, and motor units of different types insert a fibre into the same tendon organ (Binder & Osborn, 1985). That is why in physiological conditions (i.e. during normal contraction of the muscle, not selective stimulation of motor units) individual tendon organs are able to sample whole muscle force accurately (Crago, Houk & Rymer, 1982). A linear dependence between the force exerted and the firing rate of tendon organs was found with sensitivity in the range of 3.4-13 impulses.s^{-1}.N^{-1} (low force contractions in which very few units are active, are exceptions).

Lund, Richmond, Touloumis, Patry & Lamarre (1978) have reported that the jaw-closing muscles of kittens contain tendon organs. They were found in deep portions of masseter and temporalis muscles of kittens in the region of the insertion to the mandible. They are as thick as in the other muscles (30-80 μm), but rather short (100-310 μm in temporalis and 200-600 μm in masseter). The ratio of tendon organs to spindles was found to be 0.27 in temporalis and 0.17 in masseter, which is several times lower than that in other muscles. Afferents corresponding to tendon organs have not been demonstrated unambiguously in masticatory muscles with electrophysiological techniques. Because of methodological problems, the physiological properties of these receptors have not been studied in detail. Taylor (1990) concluded that tendon organs are relatively not so plentiful in jaw-closing muscles as in limb muscles, and that nothing at all is known of

their reflex connections. It should be mentioned here that masticatory system contains periodontal mechanoreceptors (see section 1.1.2.4), which can accurately sample the biting force and might partly fulfil the role of the Golgi tendon organs.

Other types of endings

Paciniform corpuscles, which are similar in structure although much smaller than the Pacinian corpuscles found in skin, are observed at the musculo-tendinous junction (Brodal, 1981; Rothwell, 1987). They are supplied by a large diameter (group II) fibre which may innervate several separate corpuscles. It is generally accepted that they are rapidly-adapting end organs with a high sensitivity to vibration. However, little work has been performed specifically on them and these receptors central connections have not been studied. Free nerve endings are found almost everywhere in skeletal muscles (Rothwell, 1987). They are rarely excited by the classical proprioceptive stimulus like stretch or vibration and are believed to be 'pressure-pain' receptors, although some of them may be more specific to humoral or metabolic stimuli.

1.1.2.2 Joint Receptors

Three main types of mechanoreceptors are found in the joints: Ruffini endings, Golgi endings similar to tendon organs, and free endings (Brodal, 1981; Rothwell, 1987). Ruffini endings consist of a small capsule which encloses a number of spray arborisations from a single afferent fibre (group II). Free nerve endings are numerous and found throughout the connective tissue and innervated by group III fibres. Initially joint receptors were thought to provide information to the CNS about static joint position (Sköglung, 1956). Later it was found that the majority of them had no response to joint angles in the middle range (Clark & Burgess, 1975). Moreover, many of them did not distinguish between extremes of flexion and extension. At present, it is generally accepted that Golgi and Ruffini endings remain silent if no stress is imposed on the capsule. Thus it has been suggested that these receptors serve to indicate extreme joint rotations, although the precise role of these receptors is still subject to discussion.

Temporomandibular joint (TMJ) receptors are potentially a significant source of proprioception. Their response to passive movements was investigated by Lund & Matthews (1981), but still little is known about their role. This is probably due to technical difficulties and the amount of surgery needed to gain access to their afferent nerves. Anaesthesia of the TMJ has been found to produce no significant changes in subjects' perception of the joint position in psychophysical experiments (Morimoto, 1990). This result is in agreement with the observation that patients with joint defects have been found to have the same discrimination ability as have normal subjects (Morimoto, 1990). Thus the role of the TMJ receptors in proprioception is presumably similar to the role of joint receptors in other parts of the body.

1.1.2.3 Skin Receptors and Receptors of the Oral Cavity

Three types of receptors are found in the skin: thermoreceptors, nociceptors and mechanoreceptors. The latter definitely play a very important role in the control of movement, especially in densely innervated regions of the hand and foot. Cutaneous mechanoreceptors are subdivided into four main types: Ruffini endings located in the dermis; Pacinian corpuscles located deeper, in the subcutaneous tissues; Merkel discs and Meissner corpuscles, found at the dermal-epidermal junction (Brodal, 1981). A series of recent studies have shown that cutaneous receptors in the fingers act to detect slipping between the skin of the finger and an object being gripped, and to initiate reflex compensatory changes in grip force (Johansson & Westling, 1984; Johansson, Riso, Häger & Bäckström ,1992; Johansson, Häger & Riso, 1992). It is believed that the major contribution to pressure sensation comes from cutaneous receptors.

The skin receptors of the human face are slowly adapting with small and well-defined receptive fields and a high degree of overlap (Johansson, Trulsson, Olsson & Westberg, 1988; Trulsson, 1993). This is also true for the receptors of lips and oral cavity (mucosal receptors). Afferents with the response properties of Pacinian corpuscles appear to be absent, but afferents corresponding to the other types of mechanoreceptors can be

identified with microneurographic techniques (Johansson *et al.*, 1988). They can be characterised by a low threshold to pressure, a high two-point discrimination and a high density reflected in large area of cortical representation.

1.1.2.4 Periodontal Mechanoreceptors

Receptors that respond when force is applied to a tooth have been loosely described as periodontal mechanoreceptors (Linden, 1990). These receptors are located in the periodontium, the tissues that invest and support the tooth. On the basis of animal studies, periodontal mechanoreceptors have been generally classified into two basic types: rapidly- and slowly-adapting. A subgroup of the latter which discharges spontaneously has been described (Anderson, Hannam & Matthews, 1970). It has been assumed that these two types of afferent response to a stimulus originate from receptors of two morphologically different groups: slowly-adapting Ruffini endings and rapidly-adapting Meissner corpuscles and other lamellated endings. However, there are no studies showing directly that afferents with these two different types of response correspond to two morphologically different types of receptors. The majority of mechanoreceptor neurons recorded peripherally had a receptive field confined to one tooth. However, some neurons have been found to respond to stimulation of up to three different teeth (Hannam, 1970).

The properties of mechanoreceptors located in the periodontal ligaments have been studied intensively over the past few years. The response characteristics of these receptors suggest that they are similar to Ruffini endings in the skin (Linden, 1990). It was noted that these receptors were situated evenly around the root of the tooth and respond maximally when the part of the ligament in which they lay was put under tension and not compression. It was also noted that the more slowly-adapting receptors appeared to be situated in the apical third of the ligament and the rapidly-adapting receptors appeared to be situated below the fulcrum of the tooth.

The directional sensitivity of human periodontal mechanoreceptors to forces applied to the teeth has been studied recently with microneurography (Trulsson, Johansson & Olsson., 1992; Trulsson, 1993). They were found to have diverse and wide receptive fields.

Periodontal mechanoreceptors contribute to the sensation of touch and pressure when forces are applied to the teeth. They can produce inhibition or excitation of the jaw-closing muscles (Brodin, Türker & Miles, 1993); therefore they may contribute to both jaw-closing and jaw-opening reflexes (see section 1.1.3 in this Chapter). They are also involved in the control of mastication (Lund, 1990). The involvement of periodontal receptors in oral kinaesthesia is apparently not critical (Morimoto, 1990).

1.1.3 Neuroanatomy and Reflex Pathways of the Masticatory System

Descriptions of the neuroanatomy of the masticatory system and its reflex pathways have been presented by Luschei & Goldberg (1981), Lund & Olsson (1983) and Kelly (1985). A detailed description has been published recently (Taylor (ed.), 1990). Figure 1.1 is a schematic diagram of the neuroanatomy of the trigeminal system and its central connections. The motor nucleus of the fifth nerve (motor nucleus of N. V) contains motoneurons innervating the masticatory muscles, which can be distinguished into γ -motoneurons and α-motoneurons. The axons of these motoneurons join with the proprioceptive afferents to form an anatomically distinct portion of the root of the fifth nerve (portio minor). As mentioned earlier (see section 1.1.2.1), no clear difference between primary and secondary muscle spindle afferent endings in masticatory muscles has been found by conventional physiological techniques. The cell bodies of the afferents innervating muscle spindles are located within the brain rather than in the semilunar (Gasserian) ganglion, which is a peripheral sensory ganglion of the trigeminal system. This collection of cells is referred to as the mesencephalic nucleus of the trigeminal nerve (Mes V or Me V). Studies of the response properties of Mes V cells by natural stimulation have shown that some cells can be activated by the stretch of jaw muscles. Others have been shown to respond to mechanical stimulation of one or more teeth, and are presumed to come from periodontal receptors. The spindle afferent cell bodies are found throughout the length of the nucleus, and those from the periodontal tissue are found in the caudal half (Cody *et al.*, 1972). Donga & Dessem (1993) have demonstrated recently that some spindle afferents project directly to the cerebellum. It is well established that the afferents of the muscle spindles of jaw-closing muscles have excitatory monosynaptic connections with the motoneurons of these muscles. The connectivity from a single spindle afferent onto homonymous motoneurons in masseter is 10-30% (Appenteng *et al.*, 1978, Nozaki, Iriki & Nokamura, 1985), which is much lower than that found in motoneurons of limb muscles, where it approaches 100% (Mendell & Henneman, 1971; Watt, Stauffer, Taylor, Reinking & Stuart, 1976).

Excitation from the same receptors presumably also comes though polysynaptic pathways that could include interneurons within the motor nucleus of N. V and in the supratrigeminal nucleus. The total pattern of reflex connections of spindles to the motoneurons appears to be different from that in the limb. Neither reciprocal Ia inhibition of motoneurons of antagonists nor recurrent (Renshaw) inhibition have been found in masseteric motoneurons (Shigenaga, Yoshida, Tsuru, Mitsukiro, Otani & Cao, 1988).

The pathways from muscle spindle afferents are involved in the jaw-jerk or **jaw-closing reflex** in jaw-closing muscles. Unlike the situation in the limbs, there is no comparable reflex response to stretch in the jaw-opening muscles, reflecting the absence of spindles in these muscles.

Oral structures, including the teeth, are innervated by afferents in the mandibular and maxillary divisions of the trigeminal nerve. The cell bodies of these afferents are located in the semilunar (Gasserian) ganglion, except for a group of periodontal and palatal mechanoreceptors that have their somata in the mesencephalic nucleus of the trigeminal nerve. Studies of some of these inputs have revealed that excitation of digastric motoneurons and inhibition of the motoneurons of jaw-closing muscles occurs at the same latency, thus giving rise to a **jaw-opening reflex.** There is a minimum of one interneuron in each pathway. Periodontal mechanoreceptors, however, can also have an excitatory effect on the motoneurons of jaw-closing muscles. This effect can change from inhibitory to excitatory with a change in stimulation parameters, i.e., with the change from quick to slow rate of force application (Brodin *et al.*, 1993).

These reflexes are adapted so that they perform useful functions in two general situations. The first is the stabilisation of the mandible when the whole body moves, and the second occurs during mastication, which is the specific function of the masticatory system. Their role seems to change accordingly in these two different conditions (Lund, Drew & Rossignol, 1984; Lund, 1990). When the animal is walking or moving the jaw-closing

reflex helps to maintain the position of the mandible relative to the maxilla and therefore acts like a postural reflex. At the same time, the jaw-opening reflex and the trigeminal-neck reflex protect the head when it contacts an unforeseen object. These reflexes cannot continue to act like this during mastication. The jaw-closing reflex must be inhibited during the jaw-opening phase. However, it can help to adjust the motor output in the jaw-closing phase of mastication, thus contributing to overcome the hardness of food. The jaw-opening reflex also changes during mastication, but it cannot be simply inhibited during the appropriate phase, because its protective component is still needed. Thus, it appears only when high-threshold receptors, or nociceptors are activated. Activation of low-threshold receptors, on the contrary, contributes to jaw-closing.

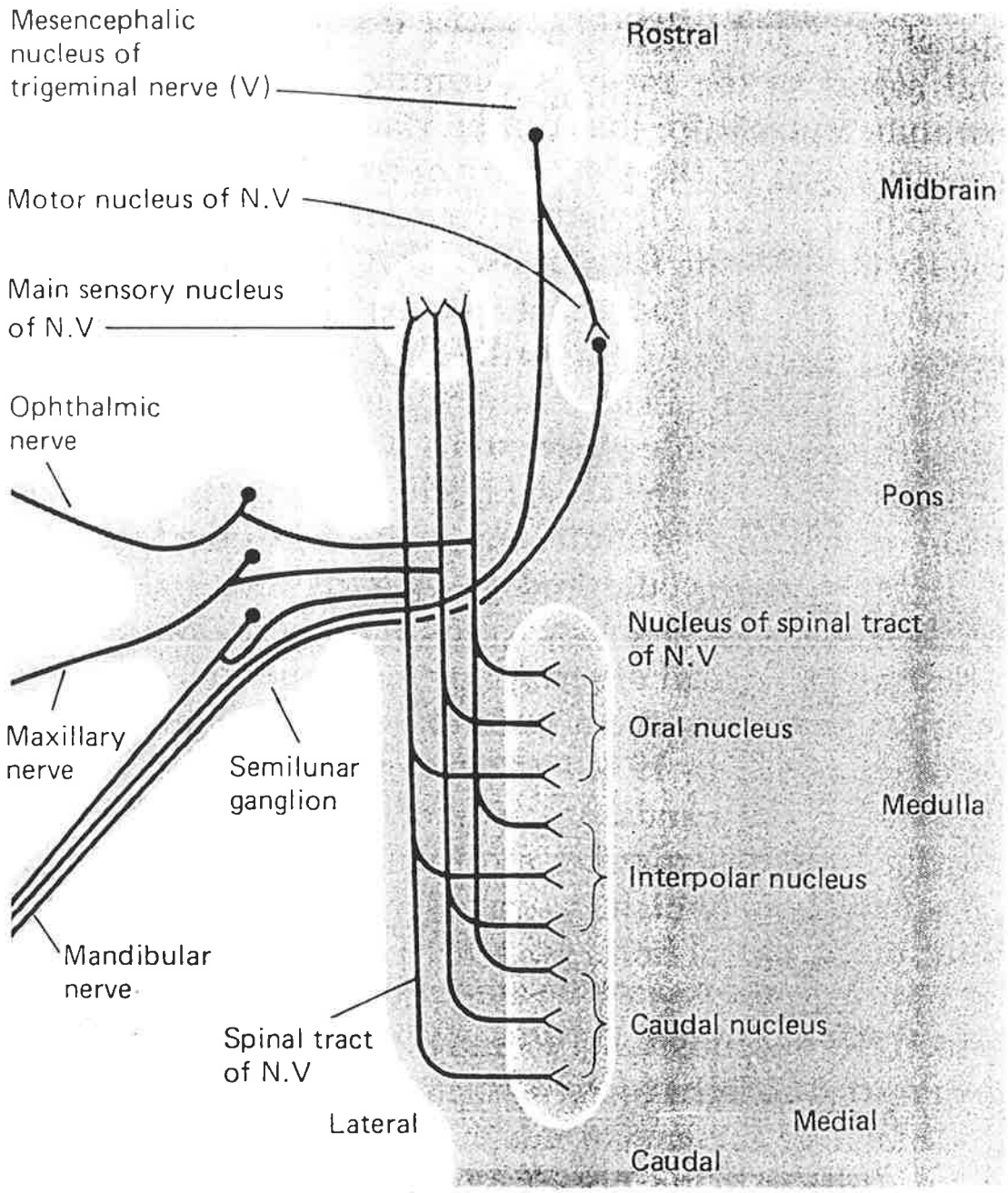

Figure 1.1 Neuroanatomy and central connections of the trigeminal nerve are shown schematically in the horizontal plane (modified from Kelly, 1985)

1.2 STRETCH REFLEX MECHANISMS

1.2.1 History of the Stretch Reflex

The phenomenon of the stretch reflex was first investigated by Liddell & Sherrington (1924) in functionally isolated muscles of decerebrate cats. Decerebration leaves intact the autogenetic reflex pathways to and from the muscle. In their experiments, Liddell and Sherrington showed that stretch, particularly of the antigravity extensor muscles, produced a reflex contraction in the muscle that opposed the applied force. The response was shown to have a dynamic component, proportional to the velocity, as well as a static component, proportional to the displacement. The decerebrate preparation has a particularly large stretch reflex because of an imbalance between descending excitatory and inhibitory systems, which produces decerebrate rigidity and affects spinal reflex mechanisms. After these experiments it became generally accepted that posture was largely maintained by the action of the stretch reflex.

1.2.2 T-reflex and H-reflex in Humans

In normal human subjects it is not possible to evoke a prolonged stretch reflex which resembles that in decerebrate cats. The simplest manifestation of the stretch reflex in humans is the so-called "T-reflex" evoked by tendon jerk. An analogous reflex can be evoked in some muscles by electrical stimulation of the nerve trunks (H- or Hoffman reflex). The H-reflex occurs because the group of Ia afferents tend to have larger diameter axons, and hence have a lower threshold for electrical stimulation than the α-motoneuron axons. Therefore, at a low stimulus intensity the Ia afferents are activated, and this can produce reflex activation of homonymous motoneurons. Traditionally these reflexes have been regarded as monosynaptic. However, taking into consideration that the durations of the rising phase of EPSP for T- and H-reflexes were found to be about 6 ms and 2 ms respectively, the possibility of contribution of polysynaptic pathways cannot be excluded (Burke, Gandevia & McKeon, 1983). Although known for a long time, T- and H-reflexes continue to be studied intensively because of their clinical significance.

There are also numerous examples of their use as a method in neurophysiological research. (e.g. Capaday & Stein, 1987, Gurfinkel, Levick & Polyakov, 1988).

The H-reflexes in human masseter muscle was first described by Godaux & Desmedt (1975a). This reflex occurred at a latency of about 5.5 ms when the subject kept his jaws tightly closed, but it could not be evoked in the relaxed muscle. The latency of the tendon jerk reflex was found to be about 7.5 ms (Lamarre & Lund, 1975). It was also shown that vibration produces not depression, but potentiation of T- and H-reflexes in masseter (Godaux & Desmedt, 1975b), in contrast to its effect in limb muscles (see also section 1.2.4 in this Chapter).

1.2.3 Long-latency Phase of the Stretch Reflex

A phasic stimulus such as a tendon tap can evoke a monosynaptic reflex response in a relaxed muscle. However, unlike in a decerebrate preparation, the reflex response to a prolonged stretch cannot be seen unless the muscle is activated. Stretch of an actively contracting muscle evokes a complex response in the surface EMG. This response can usually be classified in three phases. The first is the short-latency burst similar to the tendon jerk and is attributed to the monosynaptic reflex arc. In humans, the approximate typical latencies found in various muscles are about 25 ms in *m. flexor pollicis longus,* 41 ms in *m. flexor hallucis longus,* 7.5 ms in *m. masseter,* 13 ms in *m. infraspinatus*, 12 ms in *m. pectoralis major* and 15 ms in *m. biceps brachii* (Marsden, Merton & Morton, 1976). The last phase, which is under voluntary control of the subject, is considered to be a voluntary reaction. The middle part of the reflex is usually referred to as the long-latency phase of the stretch reflex. The stretch parameters have an important influence on both the short- and the long-latency components of the reflex (Spitzer & Claus, 1992).

Hammond (1960) studied the effect of stretch in a human muscle and found that the major force response to a stretch occurs too late to be attributed to a short-latency monosynaptic response and too early to correspond to voluntary reaction. Since then, the

long-latency component of the stretch reflex has been investigated intensively and found in many limb muscles, with various degree of prominence. As a result of these studies, it is now generally accepted that the monosynaptic pathways do not play the major role in the reaction of a limb muscles to a *slower* stretch, although they contribute to it. The major physiological response is due to the long-latency component of the reflex (Matthews, 1989).

The origin of the long-latency component of the stretch reflex is complex and could vary in different muscles. Three main possibilities should be considered in each particular case (Rothwell, 1987). Firstly, the afferent could be the same as in the tendon jerk, but impulses could traverse longer pathways (polysynaptic spinal circuits or "long-loop" routes via supraspinal structures). Secondly, the reflex could be mediated by slowly conducting afferents (for example, group II spindle afferents). Thirdly, the afferent discharge could be prolonged and/or segmented due to 'ripples' in the trajectory of the displacement. It has become accepted now that the long-latency reflex is not a purely spinal phenomenon. The contribution from the supraspinal structures is believed now to be substantial. Evidence from various sources, indicating that the long-latency stretch reflex could be mediated by transcortical pathways has been recently reviewed by Matthews (1991). Application of the novel technique of transcortical magnetic stimulation has provided new support for the transcortical hypothesis (Palmer & Ashby, 1992).

An important feature of the long-latency reflex is the adaptation of the response depending on the functional set. Hammond (1960) noticed that the reflex became larger if the subject was instructed to resist the stretch, and smaller if he was told to give way. This phenomenon has been studied by many investigators (reviewed in Houk & Rymer, 1981).

The stretch reflex pattern has usually been studied at the whole muscle level by means of surface EMG techniques. There have been only two studies examining single motor unit

responses to stretch. Calancie & Bawa (1985a, b) studied stretch-evoked responses of individual motor units in human wrist flexors, and Bawa & Tatton (1979) used monkey wrist muscles. They both found significant variability of responses of different motor units to the same stretch. In particular, they reported that higher-threshold motor units, which were not tonically active at a particular effort level, responded primarily in the long-latency phase of the reflex (Calancie & Bawa, 1985b). In the earlier article (Bawa & Tatton, 1979) it was suggested that the motoneuron pool consisted of two subpopulations, which respond differently to the stretch. The later study did not confirm this suggestion, although it did confirm the trend of higher-threshold motor units, that were not tonically active, to respond at a longer latency. However, it should be noted that these conclusions were made on the basis of very limited experimental material, a result of the difficulties of recording from identified single motor units during muscle stretch. Thus, further studies involving motor unit recording aimed at a detailed understanding of the motoneuron pool response to a stretch of the muscle are desirable for a better understanding of motor control organisation.

1.2.4 Tonic Vibration Reflex

Vibration of the muscle belly or tendon is a powerful stimulus to muscle spindle afferents and can evoke reflex activation of the muscle. This response is known as the tonic vibration reflex or TVR (Rothwell, 1987). Vibration may produce a slowly developing reflex contraction which is sustained throughout the period of vibration and which subsides slowly when vibration is stopped. Although vibration is a rhythmic stimulus, the reflex-evoked surface EMG activity appears to be asynchronous. However, spectral analysis may reveal narrow peaks at the vibration frequency and harmonics (Lebedev & Polyakov, 1992). This is due to the fact that distribution of discharges of an individual motor unit over the vibration cycle is uneven, but usually was found to be smooth with no sharp peaks (Lance, Burke & Andrews, 1973). The origin of TVR is usually assigned to polysynaptic pathways of Ia and other muscle afferents, and its slow development could be explained by their potentiation.

In contrast, vibration-evoked reflex activity in the jaw-closing muscles was found to be well synchronised with the stimulus, this being confirmed by both surface EMG and individual motor unit records (Godaux & Desmedt, 1975b). This observation has been attributed to the unique organisation of the trigeminal system and, in particular, to the absence of a reciprocal inhibitory effect from jaw-opening muscles (see section 1.1.3 in this Chapter). Thus the relative contribution of monosynaptic pathways in eliciting and maintaining of the TVR was suggested to be much stronger in comparison with limb muscles (Godaux & Desmedt, 1975b).

1.2.5 Stretch Reflexes in Human Masticatory Muscles

Despite the great interest in the stretch reflex as a manifestation of some of the underlying neural mechanisms involved in motor control, there are only a limited number of reports analysing this reflex in human masticatory muscles. The jaw-jerk reflex can be demonstrated by tapping on the chin with a neurological hammer (e.g., Godaux & Desmedt, 1975a; Murray & Klineberg, 1984). It was concluded that the short-latency excitation in the jaw-closing muscles was a monosynaptic excitation from spindles of the stretched muscles. As in the limb muscles, the amplitude of jaw-jerk reflex depended on the parameters of the displacement, and a number of other physiological factors can contribute to its modulation. Generally, the following factors should be considered: level of background muscle activity, the amount of gamma drive, the properties of intrafusal muscle fibres and pre-synaptic mechanisms (Stein & Capaday, 1988). This reflex has recently been shown to vary with the different clenching tasks (Lobbezoo, van der Glas, Buchner, van der Bilt & Bosman, 1993). Somatic and visceral sensory inputs should be able to modulate this reflex by modifying the level of activity of the reticular formation, neurones of which are known to impinge on the somata of both sensory and motor nuclei of the trigeminal system (Kuypers, 1981). It has been shown recently that the jaw-jerk reflex can be modulated by the sympathetic nervous system (Grassi, Deriu, Artusio & Passatore, 1993).

However, the pattern of displacement of the mandible may vary significantly from trial to trial when the reflex is evoked by tapping on the chin with a neurological hammer. The few studies in which a controlled stretch with a ramp and hold displacement pattern has been used include those of Lamarre & Lund (1975), Marsden, Merton & Morton (1976), Cooker, Larson & Luschei (1980) and Smith, Moore & Pratt (1985) in humans, and Goodwin, Hoffman & Luschei (1978) in monkeys. Lamarre & Lund (1975) found that displacement of the mandible by applying the stretch to incisor teeth elicits a vigorous short-latency electromyographic response in the muscles. They also showed that merely loading the mandible during a closing movement without actually stretching the muscles would elicit a similar response. Cooker *et al.* (1980) have presented evidence that the jaw stretch reflex in humans makes a significant contribution to the stability of the mandible. This effect was demonstrated directly by Goodwin *et al.* (1978) by selectively destroying the spindle afferent pathways in monkeys.

Lamarre & Lund (1975) observed a short latency (7.5 ms) response of masseter muscle to a phasic stretch and did not find any longer-latency responses after this initial, presumably monosynaptic, response. The apparent absence of any longer-latency responses was later confirmed by Goodwin *et al.* (1978) in monkeys, and Cooker *et al.* (1980) and Smith *et al* (1985) in humans. These observations are in contrast with the evidence from studies of spinal systems, where the long latency reflex was found to be far more effective in generating significant forces than the initial monosynaptic response (see section 1.2.3 in this Chapter). Indirectly this is supported by the different appearance of muscle response to vibration in the jaw-closers (see section 1.2.4), its synchronous character could be interpreted as a stronger effect of monosynaptic connections in jaw-closing muscles. This suggested that all of the physiological functions of the jaw-stretch reflex (Goodwin *et al.,* 1978, Cooker *et al.,* 1980) should be attributed to this short-latency monosynaptic pathway. Marsden *et al.* (1976), however, studied the long-latency phase of the stretch reflex systematically in a variety of human muscles. In one of their EMG records presented as "tulips", a long-latency phase of the

reflex can be seen in jaw-closing muscles. Hellsing (1988) also observed later phases of the jaw-jerk reflex as a weak deflection with a latency of about 40 ms and another burst of activity at a latency of about 70 ms.

One more inconsistency in the literature on this question is the force response of the jaw stretch reflex. Cooker *et al.* (1980) observed prominent twitch-like increase in the biting force (amplitude about 4 N), following the initial passive component due to inertia and elasticity. Goodwin *et al.* (1978) observed a qualitatively-similar pattern in monkeys. However, in the record presented by Smith *et al.* (1985), the twitch-like increase of biting force evoked by stretch appears to be absent or very small, despite their claim that their results are in good agreement with the previous two studies. In the earlier reports, the biting force was not presented (Lamarre & Lund, 1975, Marsden *et al.*, 1976,).

1.2.6 Technical Aspects of Stretch Reflex Studies in the Jaw Muscles

A better understanding of the stretch reflex in jaw muscles could be achieved if the properties of the experimental preparation and the technical problems associated with them are considered. In particular, the mechanical properties of the masticatory system differ significantly from those in many other motor systems. First, the masticatory muscles are both stiff and powerful, which means that the stretcher must be powerful to impose rapid stretches on a contracting muscle. Second, the displacements required to stretch the jaw muscles are of small amplitude. The same relative increase of the muscle length for a limb displacements of tens of millimetres is achieved by a displacement of only hundreds of microns in masticatory muscles. Third, the displacements are applied via teeth that are rigidly embedded in bone, and there is practically no soft tissue filtering of the displacement. It is likely that high frequency components or vibrations of even small amplitudes would be transmitted from the jaw bars to the muscle. This could affect the reflex response significantly, since muscle spindles are known to be very sensitive to small displacement and vibration (see section 1.1.2.1 in this Chapter). These three features of the preparation make the task of delivering a controlled lengthening to the

masticatory muscles a much more difficult mechanical problem than it is for many limb muscles.

In the studies of long-latency components of the stretch reflex in limb muscles the pattern of displacement is considered carefully and described in detail in many reports. In contrast very little information has been presented about the pattern of displacement in stretch reflex studies in masticatory muscles. The amplitude and rise time are usually the only parameters used to characterise the displacement (about 2 mm and 10 ms in Lamarre & Lund (1975); about 4 mm and 25 ms in Marsden *et al.* (1976); about 0.2 mm and 7 ms in Cooker *et al.* (1980); and about 0.5 mm and 12 ms in Smith *et al.* (1985)). This information does not characterise the stretch completely, as the transitional phases of acceleration and deceleration may still vary in duration and amplitude. Moreover, as seen in the figures (e.g. Figure 7 in Marsden *et al.*, 1976, Figure 7 in Goodwin *et al.,* 1978), the displacement was not exactly linear. The force record presented by Cooker *et al.* (1980) in Figure 1 shows that it has oscillations in the beginning which suggests that the displacement trajectory had ripples. Hence, at the onset of the present studies, there were no experimental data for stretch reflex in the masticatory system that were comparable with the studies in limb muscles in terms of the application of smooth ramp-and-hold stretches of the muscle.

The other technical problem is related to the registration of the reflex events in the masticatory muscles. It is a common practice to average the EMG responses evoked over a number of trials, using the full-wave rectified EMG signal as the input to the averager (Gassel & Ott, 1969). This method provides valuable information about reflex responses, although the method is not strictly quantitative. That is, there is no linear relationship between the rectified surface EMG average and the activity of the motoneuron pool. Furthermore, Widmer & Lund (1989) have pointed out that rectifying the EMG signal can introduce spurious peaks in the average, which may be misinterpreted as being excitatory responses. The latencies of the reflexes in masticatory muscles, proximal to the CNS, are much shorter then in many skeletal muscles. For the same reason a higher

degree of synchrony of the reflex response could be expected. These factors create the conditions where the conventional method of the averaged rectified EMG may produce significant distortions of the pattern of the reflex response (see Chapter 3). In the case of inhibition there could be a spurious, apparently excitatory peak at the reflex latency. In the case of excitation there could be disproportionately high, sometimes biphasic peaks, that overestimate the amplitude and duration of the excitatory response.

The reflex responses can be analysed quantitatively by cross-correlating the stimulus with the activity of single motor units rather than the surface EMG. This technique gives a more accurate estimate of the reflex pattern, but it is demanding (e.g., Miles, Türker & Nordstrom, 1987). It also should be considered that the results from one or several motor units may not be representative (Buller, Garnett & Stephens (1980); Garnett & Stephens, 1980), although this has not been examined for the stretch reflex in any human muscle. In particular, the reflex pattern in the higher threshold units could be different from that in lower threshold units (Datta & Stephens, 1981). Units which were not active during the background contraction could contribute to the excitatory reflex, making the overall response different from that in tonically active units. The overall reflex pattern thus could be somewhat different from that in a sample of recorded motor units.

CHAPTER 2

AN APPARATUS FOR CONTROLLED STRETCH OF HUMAN JAW-CLOSING MUSCLES

2.1 INTRODUCTION

As it has been shown in Chapter 1, there are comparatively few systematic studies of the stretch reflex in the jaw muscles. This is surprising, since there are a number of factors that make the jaw muscles a more attractive model for the study of stretch reflexes than the limbs. Firstly, the mechanics of the jaw-closing muscles are simpler than most limb muscles in that they act directly across the joint without long, in-series, elastic tendons. This enables stretches to be applied directly to the jaw muscles, and also enables changes in muscle length to be measured precisely. Secondly, the teeth are tightly secured to the jaw bones by the periodontal ligament, which allows them to move only about 250 μm with respect to the bone: thus, it is possible to apply stretches to the jaw muscles with minimal soft-tissue damping, via bite bars.

Notwithstanding these advantages, and the clinical importance of the stretch reflexes of the jaw muscles (Goodwill & O'Tauma, 1969; Ongeboer de Visser & Goor, 1974), they have not been intensively investigated. This may be because the very factors that make the jaw muscles an attractive model for studying stretch reflexes also pose some problems in the design of the muscle stretcher. Partly because they act directly across the joint, the jaw-closing muscles are both powerful and stiff, so that high forces must be applied to stretch them: this complicates the design of the control circuitry. The temporomandibular joint has a limited range of movement, and damage is readily

produced if it is over-stretched. Further, the firmness of the attachment of the teeth to the bone predisposes them to injury if rapid, powerful stretching forces are applied. Perhaps for these reasons, the stimulus in most studies of the so-called "jaw-jerk" reflex has been a tap on the chin with a tendon hammer (e.g., Godaux & Desmedt, 1975a, b; Murray & Klineberg, 1984). There are very few studies in which controlled stretch of the jaw-closing muscles has been used to evoke stretch reflexes (see sections 1.2.5 and 1.2.6 in Chapter 1).

The aim of this chapter is to describe the design and performance of the stimulator that was used to apply controlled stretches to the human jaw-closing muscles. A particularly important feature of this stimulator was the incorporation of mechanical and electronic safeguards to prevent the possibility of damage to the structures in and around the mouth.

2.2 METHODS

The basic design of the apparatus is shown in Fig. 2.1. The seated subject bites on stainless-steel jaw bars on a floor-mounted, mild-steel frame. The axis of rotation of the lower jaw bar is approximately concentric with that of the temporomandibular joint. Controlled displacements of the lower jaw bar are imposed by an electromechanical vibrator (Ling Dynamic Systems, model 406). A 200 N load cell (LC1205-K020, Litra Co. Ltd., Japan) is placed in series with the moveable probe of the vibrator to measure force, and this in turn is coupled to the lower jaw bar by a zero-backlash, rod-end bearing. It proved to be necessary to use self-centring bearings between the lower jaw bar and the frame in order to minimize friction during bites at even moderate force, e.g., less than 30% maximum bite force.

The displacement of the vibrator probe is measured with a linear-movement displacement transducer (Transtek model 0244-0000) mounted in parallel with the probe: this provides the length signal to the control circuit. An accelerometer (Intaq International model ACH-05) is mounted on the lower jaw bar to measure acceleration in the vertical plane. The adjustable mechanical stops mounted below the lower jaw bar are an important safety feature. In addition to providing absolute limits to vertical displacement of the jaw, their presence has been found to allay the concerns of some subjects about the possibility of excessive stretches.

Fig. 2.2 shows the general layout of the control system. The command signal is produced in a special-purpose computer program. This gives maximal flexibility in the design of the shape of the command signal for different experimental applications. The command signal is output through a digital-to-analogue circuit to the feedback control circuit when the computer is triggered by an external TTL pulse. The control circuit drives the power amplifier (Ling Dynamic Systems PA-500) which in turn delivers the driving current to the vibrator. A library of command templates is kept on disc.

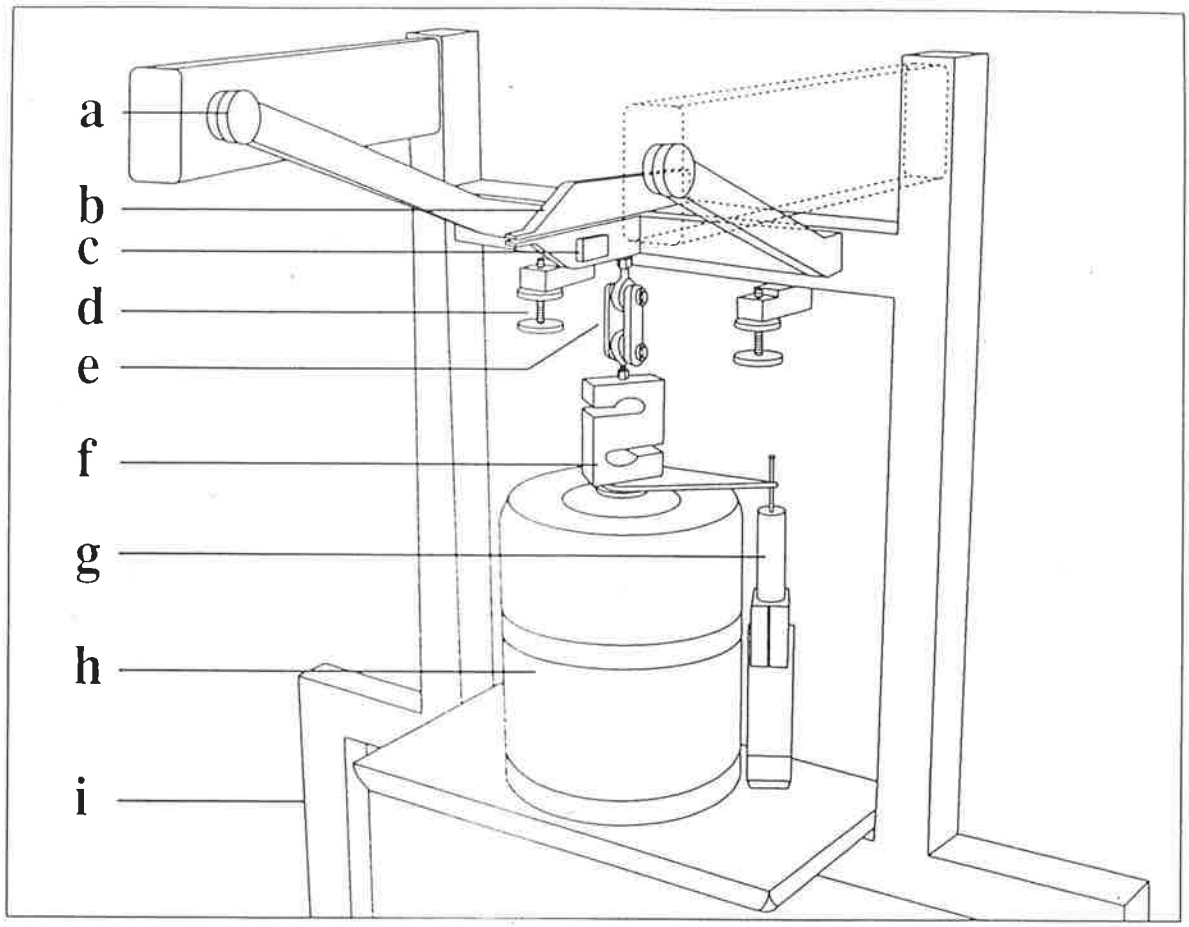

Figure 2.1 Perspective sketch of jaw-muscle stretcher. Note that dotted lines have been used selectively to show hidden parts of the system, in order that important details are not lost: for the purpose of clarity, not all hidden lines are shown by dotted lines. **a**, self-centring ball joint hinge connecting lower bite bar to frame; **b**, upper jaw bar (stainless steel); **c**, accelerometer; **d**, adjustable mechanical stop; **e**, zero-backlash, rod-end bearing; **f**, load cell; **g**, linear displacement transducer; **h**, electromagnetic vibrator; **i**, floor-mounted frame.

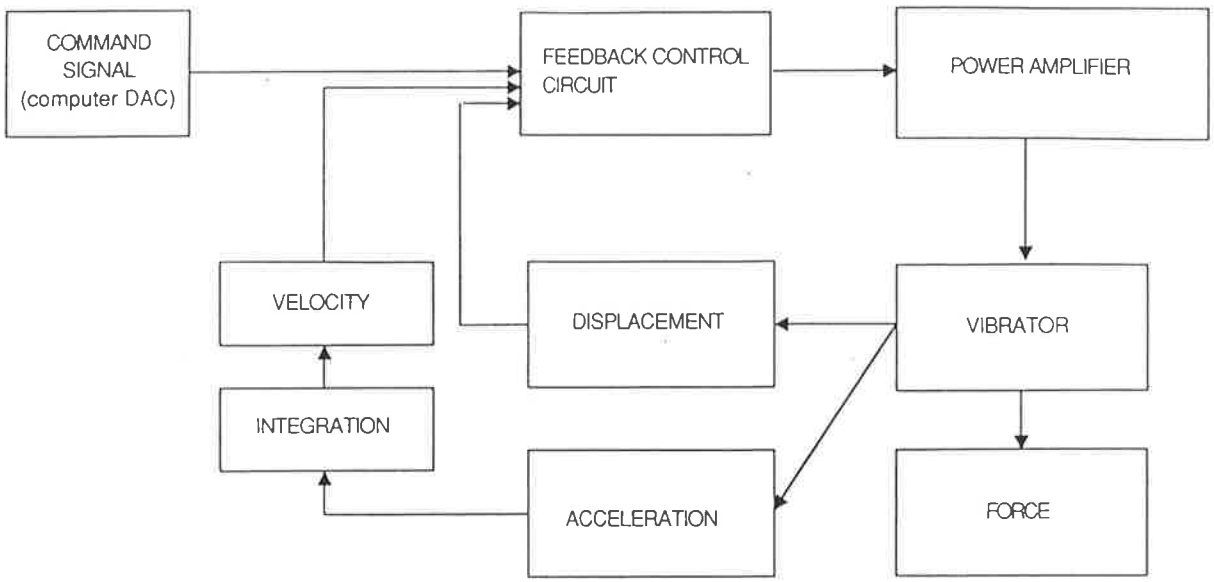

Figure 2.2 Flow diagram of jaw muscle stretcher and its control system

The so-called "interlock" facility on the PA-500 power amplifier enables the amplifier to be powered-up and powered-down smoothly. This is particularly important in preventing unwanted, possibly violent, movements of the jaw-bar in the event of a power failure, or if a lead breaks or is mistakenly disconnected. In the event of any such untoward problem, the circuit design turns the power to the vibrator off smoothly, so that the resistance to biting slowly decreases. The same facility was used to incorporated two "panic buttons" into the circuit. One of these is for the subject's use, and the other for the investigator. Touching either of these large red buttons smoothly turns off the power.

The feedback control circuit is a fairly conventional position servo, with some modifications. Firstly, provision is made for setting (adjustable) upper limits for the peak driving current, thus limiting peak stretch force. If the driving current exceeds the value set, the power amplifier is turned off smoothly through its interlock facility. Secondly, to improve the frequency response of the system, a signal proportional to the velocity of the jaw bar is summed into the control circuit. This signal was obtained by mounting an accelerometer on the lower jaw-bar, and integrating its output.

The control algorithm can be expressed as:

$$F = k_i\, d - k_j\, v \qquad (2.1)$$

where F is the feedback signal, d is the difference between the desired and actual position of the bar, v is velocity, and k_i and k_j are positive coefficients. The value of d is calculated as the difference between the control signal and output of the position sensor, and v is obtained by integration of the accelerometer output. The value of k_j was established by examining the response of the system to a step command signal for a number of values of k_j, as shown in Fig 2.3. The final value of this coefficient was chosen empirically to provide the best compromise between high frequency response and damped oscillations of the jaw bar. The displacement signal obtained with the selected value of k_j is shown by the thick dashed line in Fig. 2.3. Note that the expression $-k_j v$ is an expression of ***viscosity***: thus, the effect of increasing the value of k_j is the

electronic equivalent of adding viscosity to the system to dampen its oscillations. Fig. 2.3 shows that increases in the viscosity reduced the terminal velocity of the jaw-bar without noticeably affecting the initial acceleration. The value chosen for k_j also abolished the 33 Hz vibration of the jaw bars (i.e., the resonant frequency of the system) that could be felt through the teeth in an earlier implementation of the control circuit.

In preliminary experiments, stretches of different velocities and amplitudes were applied to the jaw-closing muscles while subjects bit with a steady, 20 N force on the jaw bars, with the help of visual feedback of the biting force. The surface electromyogram (EMG) of the right masseter muscle was rectified and averaged in the conventional way. In each experiment, the mechanical safety stops were set about 2 mm below the maximal excursion intended for the lower jaw bar, and the peak driving current was established by giving trial stretches at the maximal pre-stimulus biting force to be used with that subject. The current limiter was initially set to a low value so that the power-down circuit was tripped during the stretch, and was then progressively increased until it was set about 10% above the value at which it tripped. That is, the driving current was limited to about 110% of the maximal current required for that experiment.

The maximal rate of acceleration of the system was tested in separate experiments in several subjects with small-amplitude (1 mm) stretches, against a biting force of about 20% maximal. The bite force was fixed at this level (about 40 N for most subjects) to minimise fatigue during the 50 trials that are usually required to get a satisfactory signal-to-noise ratio in the EMG.

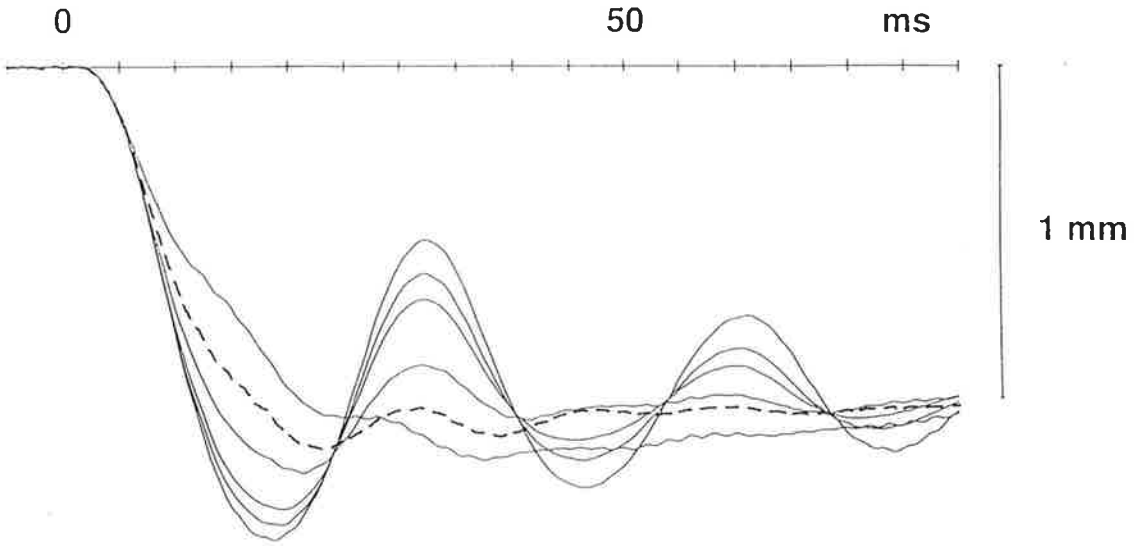

Figure 2.3 System performance at various gains of the velocity feedback signal. Each line shows the displacement of the jaw bar obtained with the same step command signal, at six different values of the parameter k_j in equation (2.1). That is, these lines show the effect of changing the gain of the velocity feedback signal to the control system, where the velocity of the jaw bar is derived from the integral of the accelerometer output. The final value chosen for the feedback is that which gave the displacement shown by the dense dashed line.

2.3 RESULTS

Data obtained in a preliminary experiment are shown in Fig. 2.4. All records are averages of data recorded in 50 trials. The uppermost record shows the command signal (dotted line) superimposed on the resulting 0.5 mm displacement of the lower jaw bar. The accelerometer output confirms that the lower bite bar accelerated smoothly to its peak velocity of 0.016 m/s in 10 ms (i.e., without "ringing"), then travelled at a constant velocity until the end of the displacement, where it decelerated with minimal overshoot. Note that the calibration bar for acceleration in this instance is 10 $m.s^{-2}$, which is about equal to g_n (gravitational acceleration). The average of the rectified EMG shows an initial excitatory peak beginning at a latency of about 9 ms from the onset of the stretch (measured from the accelerometer record), which probably represents the segmental stretch reflex excitation of the masseter muscle. This is followed by a second peak beginning at a latency of about 35 ms. During the stretch, the force applied to the teeth increased from 20 N to about 30 N. Although not shown, the shortest-latency response to stretch in this subject, when rapid stretches (equivalent to downward taps) were applied, was about 8 ms.

When subjects bit with their incisor teeth at 20 N force, the maximal acceleration of the lower jaw bar over a 2 mm displacement was about 50 $m.s^{-2}$. The peak acceleration should increase when the displacement is greater than 2 mm; however, this was not tested for reasons of safety.

Before the introduction of the tuned velocity feedback, the jaw-bar could be made to oscillate by tapping the lower jaw bar sharply with a hammer, or by setting the displacement-limiting screws so that the jaw-bar crashed down onto them during a rapid stretch. However, the combination of the "electronic viscosity" and the current-limiting circuit has made it impossible to provoke oscillations in the system.

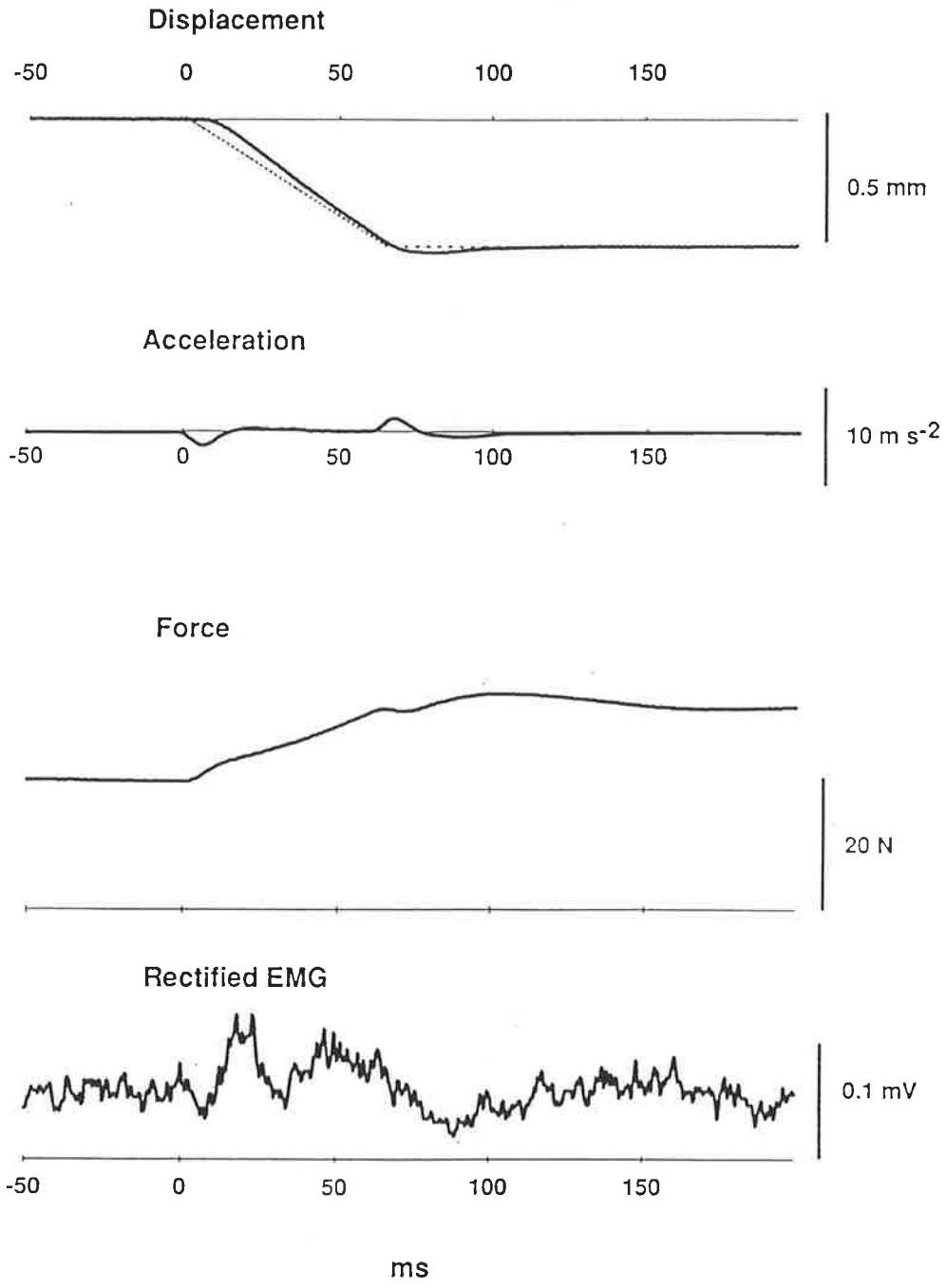

Figure 2.4 Sample results of data obtained in a reflex paradigm with the jaw-muscle stretcher. The command signal is shown as the dotted line in the uppermost graph, and is superimposed on a scaled record of the actual displacement obtained. Below this are the averages of the output of the accelerometer, and the force record obtained during 50 stretches. The lowermost record is the average of the rectified masseter EMG recorded during the same 50 trials.

2.4 DISCUSSION

The stretcher described herein incorporates several improvements over other devices that have been used to stretch the human jaw-closing muscles. For example, Lamarre & Lund (1975) used a torque motor in an open-loop control mode to displace the mandible. While this was adequate to demonstrate the existence of stretch reflexes in the jaw muscles, the present system offers an increased frequency response, substantially more power for applying rapid stretches, and improved control characteristics for producing accurate displacements under changing conditions of force. The stretcher used by Cooker *et al.* (1980) was based on a powerful vibrator, and incorporated a servo-control system: however, the force and stretch records in their Figure 1 suggest that the acceleration of the jaw bar was polyphasic.

The control circuitry of the present system was tuned to produce a monophasic pattern of acceleration of the jaw-bar (Fig. 2.4). This is particularly important in reflex studies because additional phases of acceleration may evoke additional short-latency modulations in the reflex response to stretch. The electronic damping of the jaw bar also abolished the high-frequency vibration of the lower jaw bar (at the system's resonant frequency of 33 Hz) that had been present in a preliminary version of the stretcher. Although the damping effectively increased the viscosity of the system, it did not significantly reduce the acceleration of the jaw-bar.

Fig. 2.4 shows that, in addition to improving the performance of the system, the output signal from the accelerometer also enables the latencies of the reflex responses to be determined more accurately, since the onset of the movement is often difficult to determine from the displacement signal.

Another improvement over earlier designs is that the movement of the jaw bar in the present design follows approximately the same arc as the incisor teeth during jaw movements. This stretcher is able to displace the mandible through the whole vertical

working range of the temporomandibular joint, which is about 40-60 mm at the incisor teeth for most people. Although the movement of the temporomandibular joint is not a pure rotation during jaw opening, in practice the positioning of the axis of rotation of the jaw bars near the axis of rotation of the temporomandibular joint gives a good approximation of the path of the opening movement, so that the lower incisor teeth do not slide across the jaw bar. This feature becomes particularly important when large-amplitude displacements are used.

Preliminary experiments with the stretcher indicate that its performance is ample for the investigation of stretch reflexes in human jaw muscles. The maximum acceleration that has been tested in a subject biting at 20 N force is 50 $m.s^{-2}$ (i.e., about 5 g_n). This is adequate, since the maximal downward acceleration of the jaw that will occur under normal conditions of walking and running is unlikely to exceed this value (although it is possible that higher accelerations may result from external blows to the face in accidents, etc.).

The provision of sufficient power to produce high accelerations in some experimental situations increases the risk that high forces might inadvertently be applied to the jaw bars. Hence, the incorporation of several layers of mechanical and electronic safeguards was paramount in the design of the system, and the safety of the subject is the most important consideration in its operation. In particular, it must be stressed that, in each experiment, the peak driving current to the vibrator is set to 110% of the current needed to provide the maximal acceleration required in that paradigm. If this current is exceeded for any reason, the power amplifier will turn off smoothly, and the stretch will be aborted.

CHAPTER 3

QUANTITATIVE ANALYSIS OF REFLEX RESPONSES IN THE AVERAGED SURFACE ELECTROMYOGRAM

3.1 INTRODUCTION

Surface electromyography (EMG) is widely used to study reflex phenomena in humans and animals. The object of such experiments is to determine the effect of a given stimulus on the activity of the motoneurons. Changes in the EMG signal that are time-locked to the stimulus are taken to indicate excitatory and/or inhibitory synaptic inputs to the motoneurons. In order to improve the signal-to-noise ratio, it is common practice to average the EMG responses evoked over a number of trials, using the full-wave rectified EMG signal as the input to the averager (see section 1.2.6). This method was first proposed by Gassel & Ott (1969) and it provides valuable information about reflex responses, although the method is not strictly quantitative. Furthermore, Widmer & Lund (1989) have shown that rectifying the EMG signal can introduce spurious peaks in the average, which may be misinterpreted as being excitatory responses.

Quantitative analysis of reflex responses is possible by cross-correlating the stimulus with the activity of single motor units rather than the surface EMG (see section 1.2.6), but this technique is demanding (e.g., Miles *et al.*, 1987).

The mathematical analysis of the surface EMG presented here suggests that the integral of the average of the unrectified EMG has a more direct relationship to the activity of motor units than the conventional average of the rectified EMG, and can be a useful supplement to this conventional analysis in studies of reflex responses.

3.2 METHODS AND RESULTS

3.2.1 Mathematical Analysis

Each discharge in each active motor unit contributes a small potential to the surface EMG: this will be referred to as the "surface representation" of an action potential (Miles & Türker, 1987). Surface potentials of individual motor units can occasionally be identified in the surface EMG during weak contractions, but more commonly the signal-to-noise ratio of these small potentials can only be recognised by using spike-triggered averaging from an intramuscular recording of the action potentials of a single motor unit to the surface EMG (Yemm, 1977).

The contribution of the i-th motor unit to the average of the surface EMG, $e_i(t)$, can be described analytically as the sum of $h_i(\tau)$, its surface representation waveform, when it is discharging at times t_{ik}:

$$e_i(t) = \sum_k h_i(t - t_{ik}) \tag{3.1}$$

The averaged EMG, $E(t)$, can be expressed as a sum of these contributions for all of the N active motor units:

$$E(t) = \sum_{i=1}^{N} e_i(t) \tag{3.2}$$

Let $c_i(t)$ be the cross-correlation function of the i-th motor unit's discharges with respect to repeated stimuli, i.e., the function of the unit's probability of discharge after the stimulus, in relation to the baseline value. In terms of experimental data, this is approximated by the cross-correlogram divided by the baseline value. Now $e_i(t)$ can be expressed as follows with its action potential $h_i(\tau)$ and cross-correlation function $c_i(t)$:

$$e_i(t) = \int_{-\infty}^{+\infty} c_i(t-\tau)\, h_i(\tau)\, d\tau \qquad (3.3)$$

So:

$$E(t) = \sum_{i=1}^{N} \int_{-\infty}^{+\infty} c_i(t-\tau)\, h_i(\tau)\, d\tau \qquad (3.4)$$

for N active motor units

This formula gives the most general description of the averaged post-stimulus surface EMG pattern. Both $h_i(\tau)$, the surface representation of a unit's action potential, and $c_i(t)$, the cross-correlation function of the i - th motor unit, can be estimated experimentally. However, it is possible in practice to record the action potentials of only a few of the motor units that are active in the muscle.

A simple approximation for this formula can be proposed if certain limitations are imposed on $h_i(\tau)$. In practice, the shape of the surface representations of different motor units can vary significantly depending on the location and separation of the electrodes (Yemm, 1977; Miles *et al.*, 1986; Lebedev & Polyakov, 1992). Biphasic potentials are usually observed when surface electrodes are placed along the belly of the muscle away from the end-plate zone and the tendon (Basmajian & De Luca, 1986). In this situation, the polarity of the first deflection of the action potential is the same for all motor units and depends on the polarity of the recording electrodes.

Consider the situation when the surface representations of the action potentials of all motor units are biphasic and symmetrical, but have varying amplitudes and widths. The first deflection can be considered to be positive without affecting the generality of the analysis. The time interval between the maximum and minimum of $h_i(\tau)$ is about 2-3 ms for standard surface EMG recording, while the intervals between spikes will be in the order of tens of milliseconds. Thus, the pointing approximation of the waveforms

$h_i(\tau)$ can be used. The pointing approximation for this waveform is $\sigma_i \delta'(t)$, the Dirac delta function derivative, taken with the positive coefficient σ_i.

$$\sigma_i = \int_{-\infty}^{+\infty} \tau h_i(-\tau) d\tau = -2 \int_{0}^{+\infty} \tau h_i(\tau) d\tau \qquad (3.5)$$

Now equation (3.4) can be transformed using the properties of the $\delta'(t)$ function (Hazewinkel, 1987):

$$E(t) = \sum_{i=1}^{N} \sigma_i \int_{-\infty}^{+\infty} c_i(\tau)\delta'(t-\tau)d\tau = \sum_{i=1}^{N} \sigma_i c_i'(t) = C'(t) \qquad (3.6)$$

where $c_i'(t)$ is the $c_i(t)$ derivative. $C(t)$ in this formula designates the sum of the cross-correlation functions of all motor units taken with the weights σ_i, with $C'(t)$ being its derivative. Integration of equation (3.6) gives:

$$\int_{0}^{+\infty} E(\tau) d\tau + Const = \sum_{i=1}^{N} \sigma_i c(t) = C(t) \qquad (3.7)$$

This shows that integrating the averaged EMG $E(t)$ approximates the averaged cross-correlation function $C(t)$, when the motor units cross-correlation functions are summed with certain weights σ_i. Consider now the meaning of these coefficients. Equation (3.5) shows that the coefficient σ_i will grow proportionally with the increase of amplitude of the surface representation $h_i(\tau)$. If the amplitude is fixed and the time scaling of the waveform is changed, the coefficient will change in proportion to the second power of the duration of the waveform.

3.2.2 Experimental Data

This theoretically-obtained relationship was tested against experimental data. An intramuscular electrode was inserted into the human masseter to record the activity of two simultaneously-active motor units. The unit potentials were discriminated with a computer-based system (SPS-8701). The surface EMG was recorded with electrodes placed away from the end-plate area. With the help of visual feedback of frequency, the subject ran one of the motor units at a constant mean rate of about 10 Hz while brisk, 1 N taps were applied to an ipsilateral incisor tooth (Bjørnland, Brodin & Aars, 1991).

The reflex response to 200 stimuli was determined by averaging the EMG both with and without full-wave rectification (Fig. 3.1A, upper and middle traces). The EMG bandwidth used in the analyses was 20-1,000 Hz. The integral of the average of the unrectified EMG is shown in the lowest trace: this integral is described by the left side of equation (3.7). The spike trains of the 2 motor units were cross-correlated with the stimuli (Fig. 3.1C, upper and middle traces) and then summed (lowest trace in Fig. 3.1C) to give an estimate of the right side of equation (3.7). The surface representations of units 1 and 2, obtained by spike-triggered averaging of the unrectified surface EMG (N>2,000 triggers), are shown in Fig. 3.1B with the same time and voltage scaling as the averaged EMGs. The surface representations of both units are biphasic and nearly symmetrical, which justifies the approximation proposed in equation (3.7).

The initial response in the average of the rectified EMG (Fig. 3.1A, upper trace) is a trough commencing at a latency of about 15 ms, signifying an inhibitory input to the motoneurons. The duration of the trough is about 20 ms. Following this is a peak lasting about 15 ms, after which the record returns to the pre-stimulus level. The principal features of the average of the unrectified EMG are a much-briefer trough at about 15 ms latency, followed by a positive peak beginning at about 35 ms. The shape of the integral of the unrectified average is broadly similar to the shape of the rectified average.

The cross-correlograms of the activity of the two motor units (Fig. 3.1C) show that they ceased firing abruptly, 20 ms after the stimulus. With the exception of one action potential in unit 2 at 30 ms, both units remained completely silent for 16 ms, after which unit 1 resumed firing at about the pre-stimulus rate, and unit 2 discharged a few additional spikes at a rate above the mean pre-stimulus frequency. The cross-correlograms of the other motor units contributing to the surface EMG are not known, but if it is assumed that these two are representative, their sum (Fig. 3.1C, bottom) can be taken as an estimate of $C(t)$, the right side of equation (3.7).

The shape of the summed cross-correlogram closely resembles that of the integral of the averaged EMG. In particular, the width of the inhibitory trough and the following peak match closely. The initial trough in the averaged rectified EMG is slightly narrower than the trough in the cross-correlograms, because of the more gradual slopes of the sides of the trough. The width of the peak centred at about 50 ms in the cross-correlograms is also accurately reflected in the integral, whereas it appears relatively wider in the averaged rectified EMG (Fig. 3.1A, lowest trace).

Data from a slightly different experimental paradigm are shown in Fig. 3.2, in which I recorded the reflex response in human masseter to moderate electrical shocks applied to the lip. The uppermost record shows that these stimuli evoked the well-known, biphasic inhibitory response in the average of the rectified masseter EMG (Miles & Türker, 1987): however, the average contains a peak (arrow) immediately before the first inhibitory trough (*c.f.* Widmer & Lund, 1989).

The shape of the integral differs in two important respects from that of the rectified average. Firstly, the peak preceding the first trough is absent in the integral; and secondly, the peak that separates the first and second troughs does not reach the pre-stimulus level in the integral, although it overshoots the pre-stimulus level significantly in the rectified average.

The lowermost trace in Fig. 3.2 shows the integral of the averaged EMG superimposed on the average of the rectified EMG. An arbitrary constant was chosen for the calculation of the integral so that its pre-stimulus level and minimal value matched those of the averaged rectified EMG (see equation (3.7)). This figure shows that the first peak in the average of the rectified signal corresponds with the beginning of the inhibitory trough shown in the integral. The amplitude of the peak at about 35 ms latency in the average of the rectified signal is nearly twice that of the background level. In the integral however, the peak does not reach the level of the background activity.

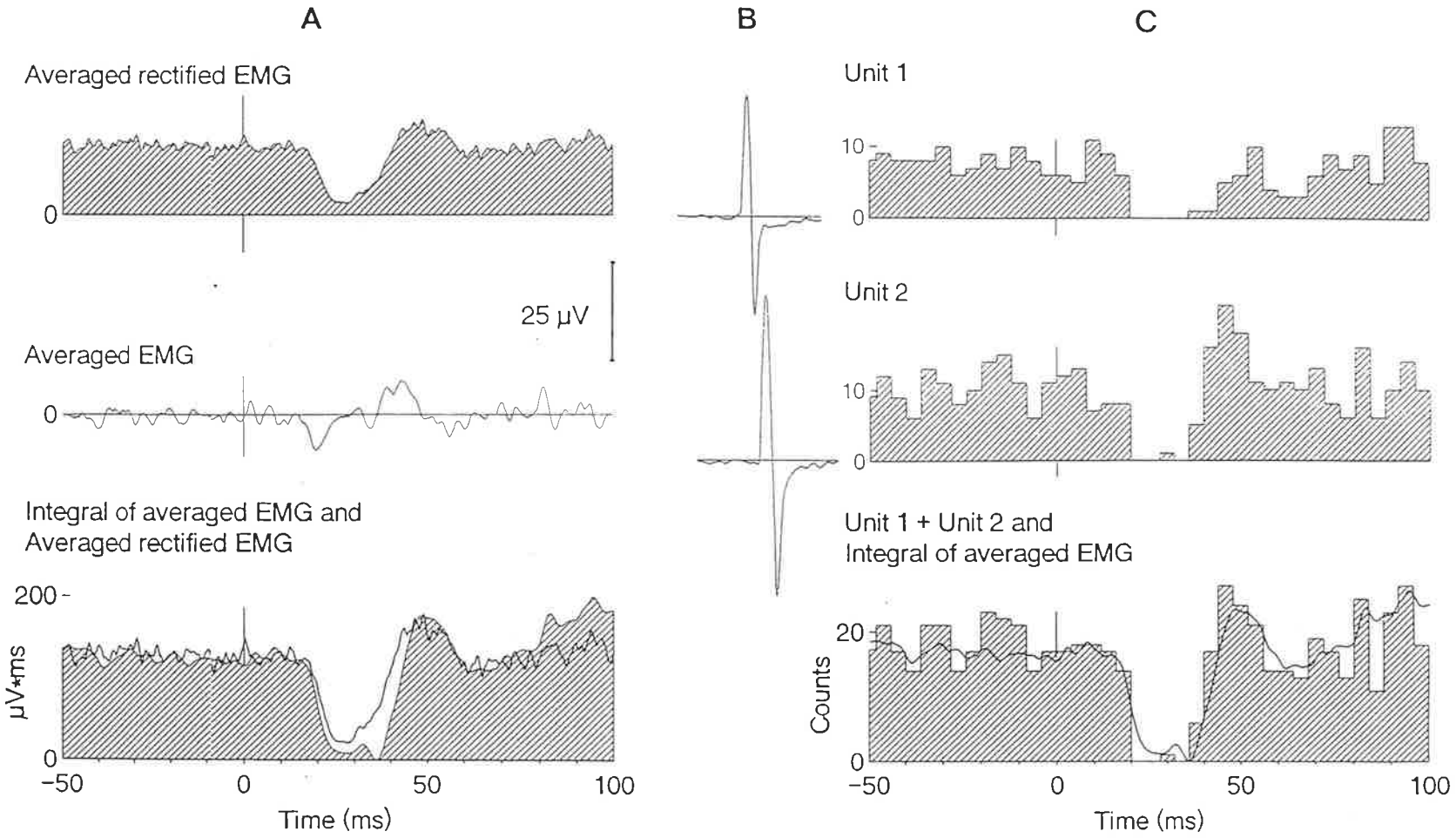

Figure 3.1. Analyses of the reflex response evoked in human masseter by tooth-taps: averages of 200 trials. **A**: *upper trace*, average of the full-wave rectified EMG; *middle trace*, average of the unrectified surface EMG; *lower trace*, integral of the unrectified, averaged EMG (shaded) with average of rectified EMG superimposed. **B**: surface representations of the two motor units whose histograms are shown in B, obtained by spike-triggered averaging of the surface EMG. Same voltage and time scales as used in A. **C**: *upper and middle traces*, peristimulus time histograms (PSTHs) of two separate motor units, recorded with an intramuscular electrode; *lower trace*, the sum of the two PSTHs above, with the integral of the unrectified average superimposed on it.

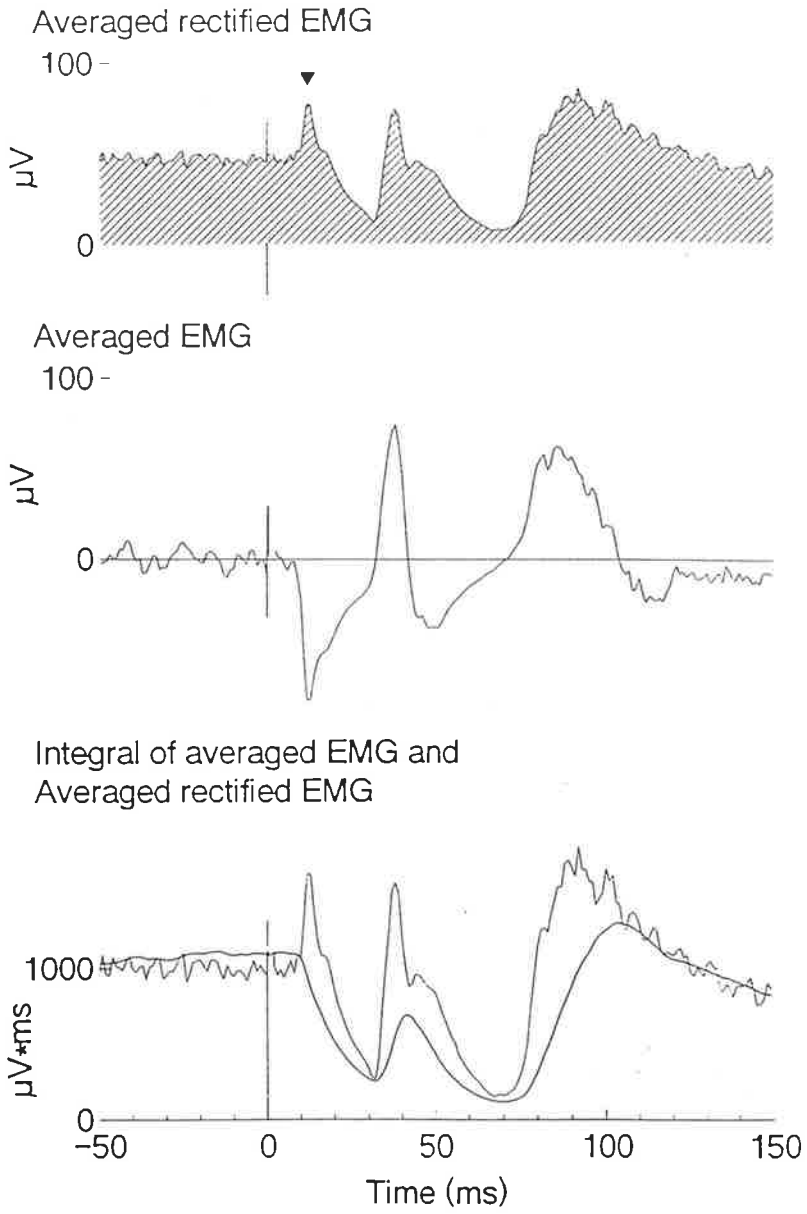

Figure 3.2. Analyses of the reflex response evoked in human masseter by moderate electrical lip-shocks: averages of 250 trials. *Upper trace*, average of the full-wave rectified EMG; *middle trace*, average of the unrectified surface EMG; *lower trace*, integral of the unrectified, averaged EMG superimposed on the average of the full-wave rectified EMG. Note that the stimulus artefact in the averaged EMG signal was deleted before integrating. The arrow shows the spurious peak in the average of the rectified EMG.

3.3 DISCUSSION

The conventional method for examining reflex responses in humans is to record the stimulus-locked average of the rectified surface EMG (Gassel & Ott, 1969). However, there is no firm mathematical basis for making quantitative analyses of neuronal activity from averages obtained in this way. Moreover, Widmer & Lund (1989) have demonstrated convincingly that the process of rectifying the EMG signal can result in a peak immediately before a sharp inhibitory trough, an example of which is shown in the Fig. 3.2 (arrow). Peaks such as these have in the past been attributed wrongly to excitatory reflexes. Here I have described a novel approach to the analysis of the surface EMG. Mathematical analysis (see section 3.2.1) shows that the integral of the average of the *unrectified* EMG reflects the activity of motor units, providing that the surface representation of the units' activity is biphasic and fairly symmetrical. This analysis offers several advantages over the normal rectified averaging procedure.

Firstly, unlike the rectified average, the integral of the averaged EMG can be related linearly to the activity of the motor units in the muscle. This enables stimulus-evoked changes in motor unit activity to be measured quantitatively. Consider, for example, the peak occurring at 35 ms latency in Fig. 3.2. In the average of the rectified EMG, this peak is 60% higher than the pre-stimulus level, whereas in the integral it remains well below it, indicating that the motoneuronal activity remains depressed. It is likely, therefore, that the average of the rectified EMG overestimates the motoneuronal activity at this latency in this example.

Secondly, the duration of peaks and troughs in the integral accurately reflect the overall pattern of activity of the motor units. In contrast, the duration of peaks and troughs on the rectified averaged EMG consistently overestimates the duration of bursts and underestimates the duration of lulls in motor unit activity, as shown in Fig. 3.1, 3.2.

Thirdly, use of the integral will prevent artefacts and consequent errors of interpretation that are induced by the rectification process, such as that shown in Fig. 3.2 (arrow).

Finally, the integration procedure by its very nature results in a smoother record than is obtained by averaging rectified signals.

Although the integral of the unrectified average has some significant advantages over the conventional average of the rectified EMG for the analysis of reflexes, there are certain limitations on its use. The integral should be used only when the surface representations of the motor unit potentials are biphasic and symmetrical. This can usually be achieved when both surface electrodes are placed along the belly of the muscle, on the same side of the end-plate region (i.e., the motor point). For easy comparison with conventional averages, the polarity of the electrodes should be arranged so that the first deflection in the EMG signal is positive-going. This can be achieved by examination of the surface EMG during weak contractions, when only a few units are active.

The second limitation is that, unlike the conventional average, the integral does not automatically indicate the level at which the muscle is completely inactive: this level is determined arbitrarily by the value assigned to *Const* in equation (3.7). I have found that it is convenient to adjust the integral so that it matches the level of mean pre-stimulus activity and the point of minimal activity in the corresponding averaged rectified EMG signal, as shown in Fig. 3.2. When this is done, quantitative comparisons of the amount of reflex activity evoked by different stimuli can be made when the level of pre-stimulus EMG activity is kept constant.

It is sometimes not recognised that the bandwidth of the raw EMG signal can affect the bandwidth of the average; it can therefore, of course, also affect the shape of the integral. In particular, excessive high-pass filtering of the EMG signal before it is averaged can change the shape and amplitude of low-frequency waves in the resulting average, and hence the integral. Thus, the time constant (1/frequency) of the high-pass filter applied to

the raw EMG signal should be much higher than the latencies of the reflex responses that are being investigated. A high-pass filter set at 1-2 Hz should not introduce any distortions in the reflex patterns with latencies of less than 100 ms.

Finally, the integral is sensitive to mechanical and electrical artefacts.

It is clear from the foregoing that the new analysis of integrating of the averaged EMG should be used to complement the conventional averaging technique in reflex studies, rather than to replace it. Its advantages are that the timing of neuronal activity can be measured with increased accuracy, and differences in the amplitudes of responses to different stimuli can be measured quantitatively.

Finally, the mathematical approach that I have applied to the quantitative analysis of the EMG in the present Chapter is equally applicable to the quantitative analysis of neurograms. This may be of particular interest when quantitative estimates of the amount of neural traffic in peripheral nerves are sought under changing experimental conditions, in both human and animal experiments.

CHAPTER 4

STRETCH REFLEXES IN HUMAN MASSETER

4.1 INTRODUCTION

The characteristic response of most limb muscles to stretch is an initial burst of excitation at monosynaptic latency, followed by a more sustained excitation at longer latency. The longer-latency response is believed to be partly the result of a transcortical pathway (Marsden *et al.*, 1976, Wiesendanger & Miles, 1982; Matthews, 1991). However, earlier investigations of the stretch reflexes of the jaw-closing muscles have concluded that, in contrast to most limb muscles, the human jaw-closing muscles respond to stretch only with a short-latency excitatory reflex (see section 1.2.5 in Chapter 1). In most investigations of the stretch reflexes of the jaw muscles, the stimulus has been a tap on the chin with a tendon hammer to elicit the so-called "jaw-jerk" reflex (e.g., Godaux & Desmedt, 1975a; Murray & Klineberg, 1984). The few studies in which controlled stretch of the jaw-closing muscles has been used to evoke stretch reflexes include those of Lamarre & Lund (1975), Marsden, Merton & Morton (1976) and Cooker, Larson & Luschei (1980) in humans, and Goodwin, Hoffman & Luschei (1978) in monkeys.

If the jaw-closing muscles lack a long-latency reflex response to stretch, this is an intriguing difference between the limb and the jaw muscles, especially as it is the long-latency component of the stretch reflex that is believed to have the greater functional significance (see Rothwell, 1987; Matthews, 1991 for review). In particular, it provides a coordinated muscle response to stretch (Gielen, Ramackers & van Auylen, 1988). This issue was re-investigated and the results are presented in this chapter.

4.2 METHODS

The experiments were conducted with the approval of the Committee on the Ethics of Human Experimentation at The University of Adelaide. The subjects were 10 volunteers, aged 18-46, including the author, who gave informed consent, and participated in 20 experiments.

Subjects were seated comfortably so that they could bite with their incisor teeth on a purpose-built, jaw-muscle stretcher. The stretcher is described in detail in Chapter 2 (see also Miles, Poliakov & Flavel, 1993). The baseline jaw separation was determined by the thickness of the jaw-bars: for stretches, the incisal surfaces of the teeth were about 3 mm apart, and for the unloading stimuli, they were initially 4 mm apart. The biting force was measured with strain gauges mounted near the teeth on the lower jaw-bar, and the vertical acceleration of the lower jaw-bar was measured with an accelerometer.

Surface electrodes were placed on the skin overlying the right masseter muscle to monitor its electrical activity (bandwidth 2-1000 Hz). The two electrodes were placed approximately in line with the direction of the muscle fibres. One electrode was at the level of the lower border of the mandible, and the other 25 mm above this, close to the motor point. Preliminary experiments showed that, with this placement, the waveform obtained by triggering an average of the surface EMG on the spikes of a single motor unit in masseter was approximately symmetrical (see Chapter 3 and Poliakov & Miles, 1992). This symmetry is reflected in the averaged EMG response to a jaw-jerk stimulus (e.g., the unrectified average in Fig. 4.1A). During each recording run, the subject maintained a steady biting force of about 10% of the maximal voluntary biting force (MVC) using visual feedback of the output of the force transducer on the bite bars. Each run consisted of 50 randomly-timed trials, in which the stimulus was a controlled displacement of the mandible. Stimuli were given at intervals of not less than 1.4 s. The characteristics of the ramp displacements (duration, velocity, rise-time, etc.) were specified in a special-purpose program on a personal computer, and output to the control circuitry

through a digital-to-analogue circuit. The amplitudes of the displacements were 0.5 and 1.0 mm. Unloading stimuli with the same profiles (inverted) were also given in different runs. In addition to the ramp stretches, the responses to displacements produced by square wave command signals of 1 ms duration were measured. This was to produce high jaw accelerations similar to those in the jaw jerks elicited in most earlier experimental studies, as well as in clinical practice, by tapping on the chin with a tendon hammer.

The electromyogram (EMG), displacement, force and acceleration signals were recorded on digital tape. Both the full-wave rectified and the unrectified masseter EMG were averaged (sampling rate 2 kHz per channel, 12 bits resolution). A mathematical analysis of the EMG presented in Chapter 3 has shown that, subject to some constraints, the integral of the average of the unrectified EMG mirrors the underlying motor unit activity; i.e., it resembles in shape the combined peristimulus time histograms (PSTHs) of the active units (see also Poliakov & Miles, 1992). This analysis is ideally used in conjunction with the conventional average of the full-wave rectified signal. The integral of the averaged EMG avoids spurious and/or exaggerated peaks and troughs which can appear in the latter. Both analyses were computed off-line. The displacement, force and acceleration signals were also averaged off-line. All response latencies were measured from the onset of jaw displacement, manifest as the first deflection in the acceleration trace, to points of sharp inflexion on the integral of the averaged EMG. These inflexions usually corresponded to those in the average of the rectified record occurring at similar times, although differences were often observed in the relative size of peaks and troughs in the two analyses. In order to determine whether the mechanoreceptors in the periodontal ligament were contributing to the response (*c.f.* Brodin, Türker & Miles, 1993), the reflex response to stretch was recorded before and after these receptors were blocked by infiltration of local anaesthesia (2% lignocaine with adrenalin 1:80,000) around the roots of the incisor teeth in 2 subjects.

4.3 RESULTS

The results of delivering a weak jaw-jerk stimulus by sending brief, square-wave command signals to the stretcher are shown in Fig. 4.1A. In this example, the jaw was displaced about 0.01-0.02 mm downwards. (The amplitudes of such small displacements could not be measured precisely). The acceleration record shows that this stimulus produced a brief (10 ms) burst of vibration in the jaw bars: the force record suggests that some of this vibration was transmitted to the teeth and to the jaw muscles. This stimulus, which was described by the subject as a very weak tap on the teeth, evoked a prominent reflex excitation of the masseter at 9 ms latency, which was followed by a period of reduced activity of about 16 ms duration. The average of the full-wave rectified EMG shows that the amplitude of the response was several times the background excitation level, while the average of the unrectified signal emphasises the highly synchronous nature of the discharge; i.e., it is closely analogous to the response to the so-called "tendon jerk" in limb and jaw muscles. With graded stimuli producing peak accelerations of 0.3-1 $m.s^{-2}$, the peak amplitude of the earliest EMG response, measured from the integral of the unrectified average, increased approximately in proportion to the peak acceleration of the stretch.

The pattern of masseter response to a long, slow stretch in the same subject is shown in Fig. 4.1B. The accelerometer record shows that the jaw bar accelerated smoothly at the beginning and end of the 64 ms ramp stretch, and did not vibrate during the ramp phase. The pattern of the reflex response is evident in the averages of both the rectified and the unrectified EMG signals. The initial phase of the response was excitation which began at about 10-12 ms latency and lasted for about 10 ms. Following this initial peak, the activity fell slightly below the baseline, indicating the presence of a so-called "silent period". A second peak of excitation then began at about 35-40 ms, and continued until it was strongly depressed at about 70-75 ms, following deceleration with the short-latency delay.

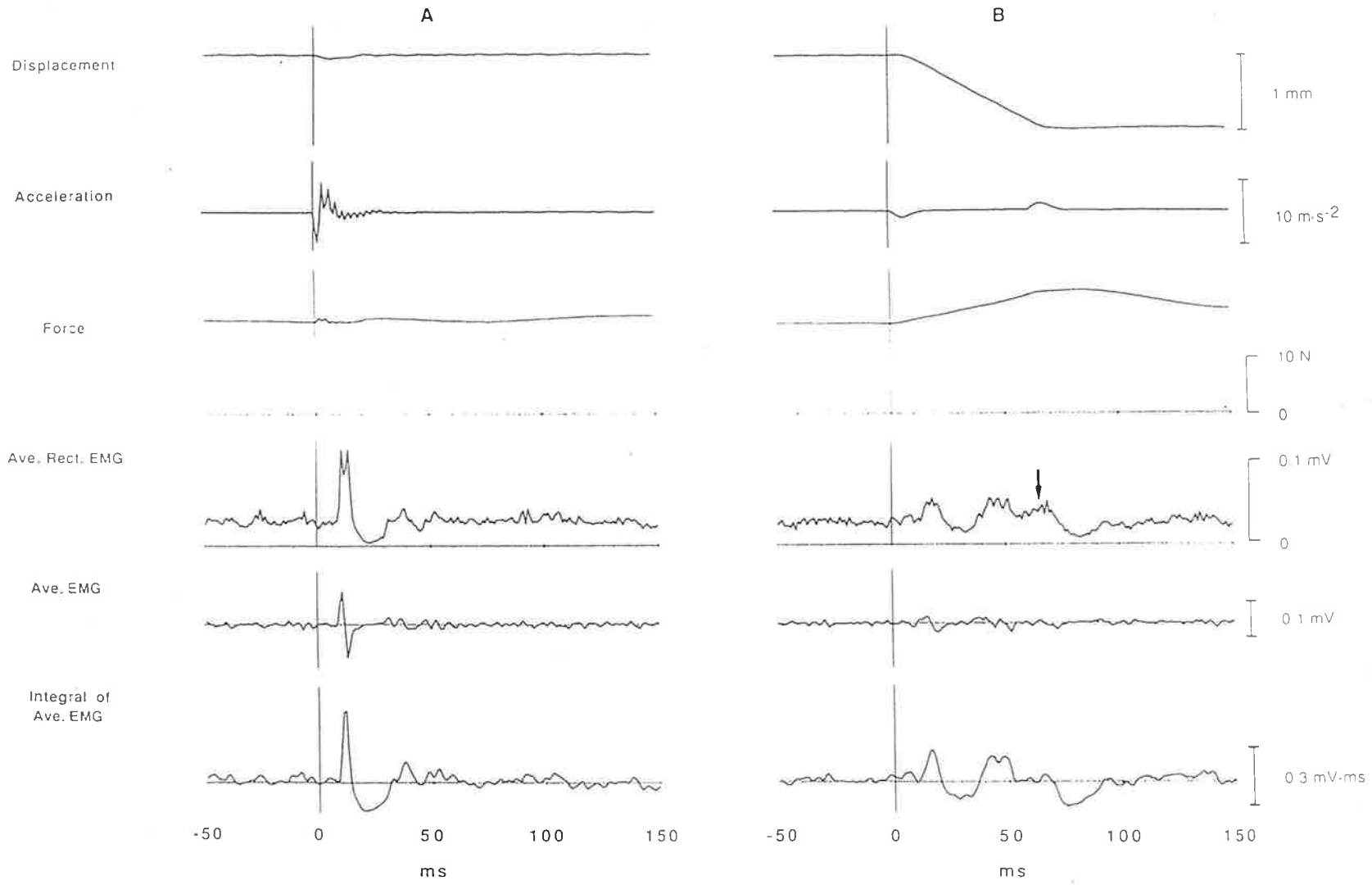

Figure 4.1 The reflex response of the human masseter to mechanical stimuli during an isometric bite at about 10% MVC. **A**, a simulated jaw-jerk, produced by sending a 1-ms square-wave command signal to the stretcher. **B**, Responses of the same subject to a 1 mm stretch of the jaw-closing muscles, at 0.015 $m.s^{-1}$. The vertical arrow shows the time at which the deceleration of the jaw-bar began.

Thus, there were two distinct phases of excitation, and two periods in which the muscle activity fell below the prestimulus level. The short-latency excitation is clearly the conventional, probably monosynaptic, segmental reflex response. Several factors may be involved in the depression in the baseline of the integral that follows this peak. Firstly, the integral reflects the shape of the PSTH of the motoneuronal activity (Poliakov & Miles, 1992), and the effect of an excitatory post-synaptic potential (EPSP) in the parent motoneuron is to move forward in time the unit potentials that would otherwise have occurred: this produces a trough in the PSTH following the excitatory peak (Miles, Türker & Le, 1989). The initial depression in the integral is due at least partly to this phenomenon. Secondly, it is known that tapping on teeth produces a predominantly inhibitory reflex response in masseter at about this latency (Brodin *et al.*, 1993). Hence, it is possible that activation of the mechanoreceptors around the teeth may have contributed to the pattern of reflex responses evoked by this stimulus, including this initial depression. However, blocking of the afferents from the receptors around the incisor teeth with local anaesthetic had a negligible effect on the overall pattern of the reflex response in the two subjects in which it was tested.

Thirdly, the integral of the average of the unrectified EMG in Fig. 4.1B shows that the record fell below the baseline about 10 ms after the onset of deceleration of the stretch (arrow). This is shown even more clearly for stretches of different duration in Fig. 4.3. This point was examined further by averaging the reflex response to unloading the masseter during an isometric bite, by moving the jaw bar rapidly upwards. Fig. 4.2 shows that this stimulus also elicited a reflex decrease in masseter activity after about 10 ms, which is comparable with the latency of the short-latency stretch reflex. Thus, in Fig. 4.1B, it is likely that the spindles continued to fire throughout the 64 ms ramp stretch, contributing to the longer-latency excitatory response; then, when the ramp finished, the reduced spindle excitation during the hold phase led to disfacilitation of the parent motoneurons.

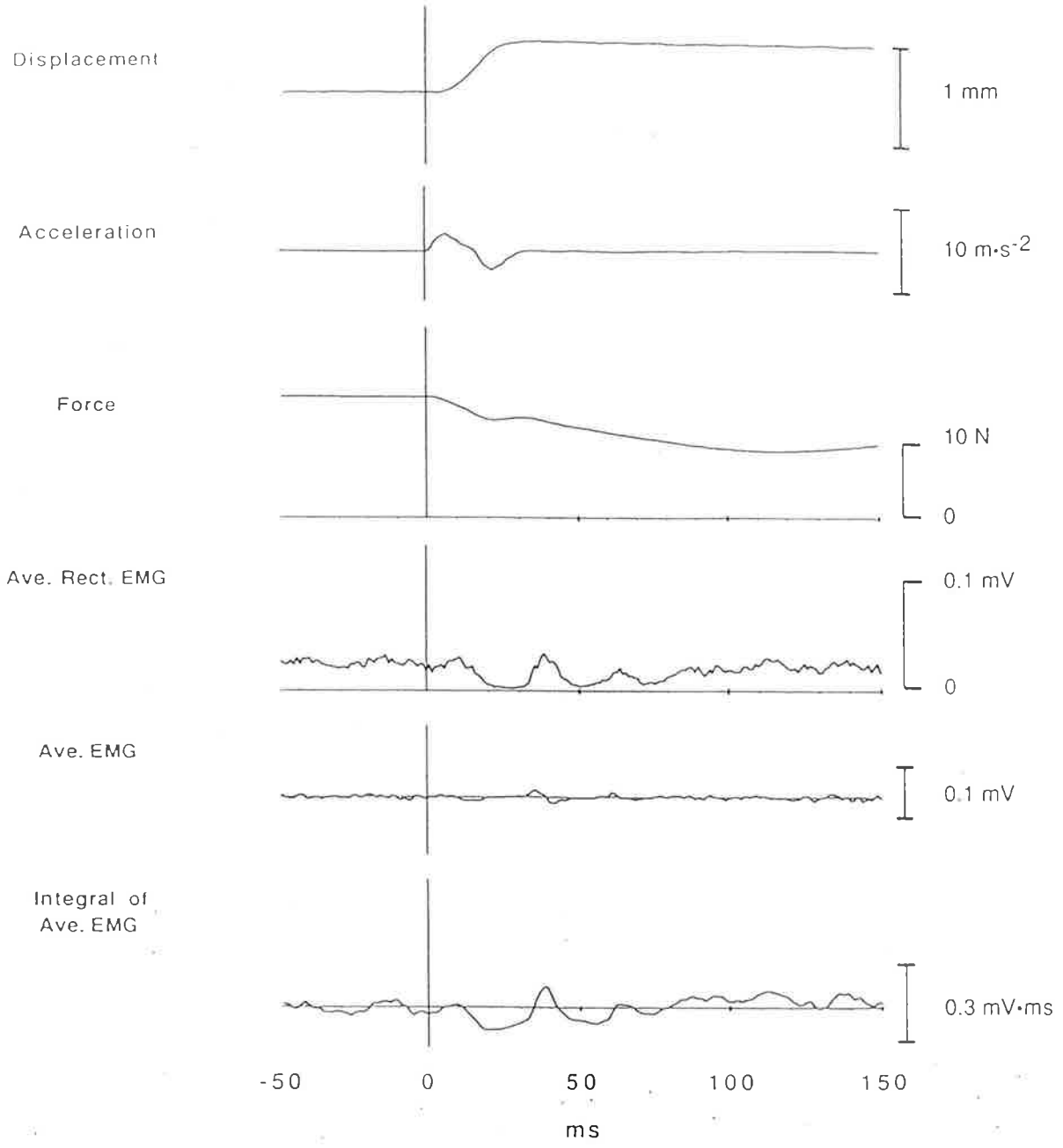

Figure 4.2 Reflex response of masseter to unloading of the jaw-closing muscles. The jaw-bar was moved 0.5 mm upwards at 0.03 $m.s^{-1}$ during an isometric bite at about 10% MVC. Same format as Fig. 4.1. Note that the initial fall in biting force due to reduced stretch of the elastic elements in the muscle is followed by a second, longer-latency force decrease which is the result of disfacilitation of the jaw-closing muscles.

The pattern of reflex responses of one subject to stretches of different lengths and velocities is shown in Fig. 4.3. The vertical arrows show the time of onset of deceleration (as in Fig. 4.1B). Consider first the longest-duration, 1-mm stretch (A). The initial response was the classical monosynaptic response, beginning at 11 ms latency. The second, more-prolonged burst of excitation began at about 40 ms, and was terminated by disfacilitation 10 ms after the jaw-bars begin to decelerate (arrow). When the velocity of the 1 mm stretch was increased to 0.03 $m.s^{-1}$ (B), the amplitude of the monosynaptic response increased. A peak beginning at about 40 ms latency is still visible in B, but is segmented into two. The trough between the two peaks is the result of disfacilitation from the deceleration of the jaw-bar. The fastest stretch (C) evoked a still-larger amplitude monosynaptic response, while the long-latency peak was almost totally suppressed.

The velocities of the 0.5 mm stretches in Fig. 4.3D-F were the same as those in the corresponding 1-mm stretches (A-C). Despite this, the amplitudes of the monosynaptic reflexes were greater for the longer stretches in this subject. This is presumably due to the different level of pre-stimulus EMG activity. When the data from all subjects were considered, the relative amplitude of the monosynaptic response was found to depend only on the initial acceleration, and not on the length of stretch.

The shape of the responses to the 0.5 mm stretches was also complicated by disfacilitation. In Fig. 4.3D, a trough separates the small, brief peak beginning at about 40 ms and the larger peak at 55 ms. This was again the result of disfacilitation; that is, when a stretch of the same velocity was prolonged as in A, the long-latency component of the reflex excitation was no longer segmented. In E, the disfacilitation (beginning 10 ms after the arrow) delayed the onset of the long-latency response until 60 ms, while in F, the disfacilitation began so early that the integral was kept below baseline until about 80 ms.

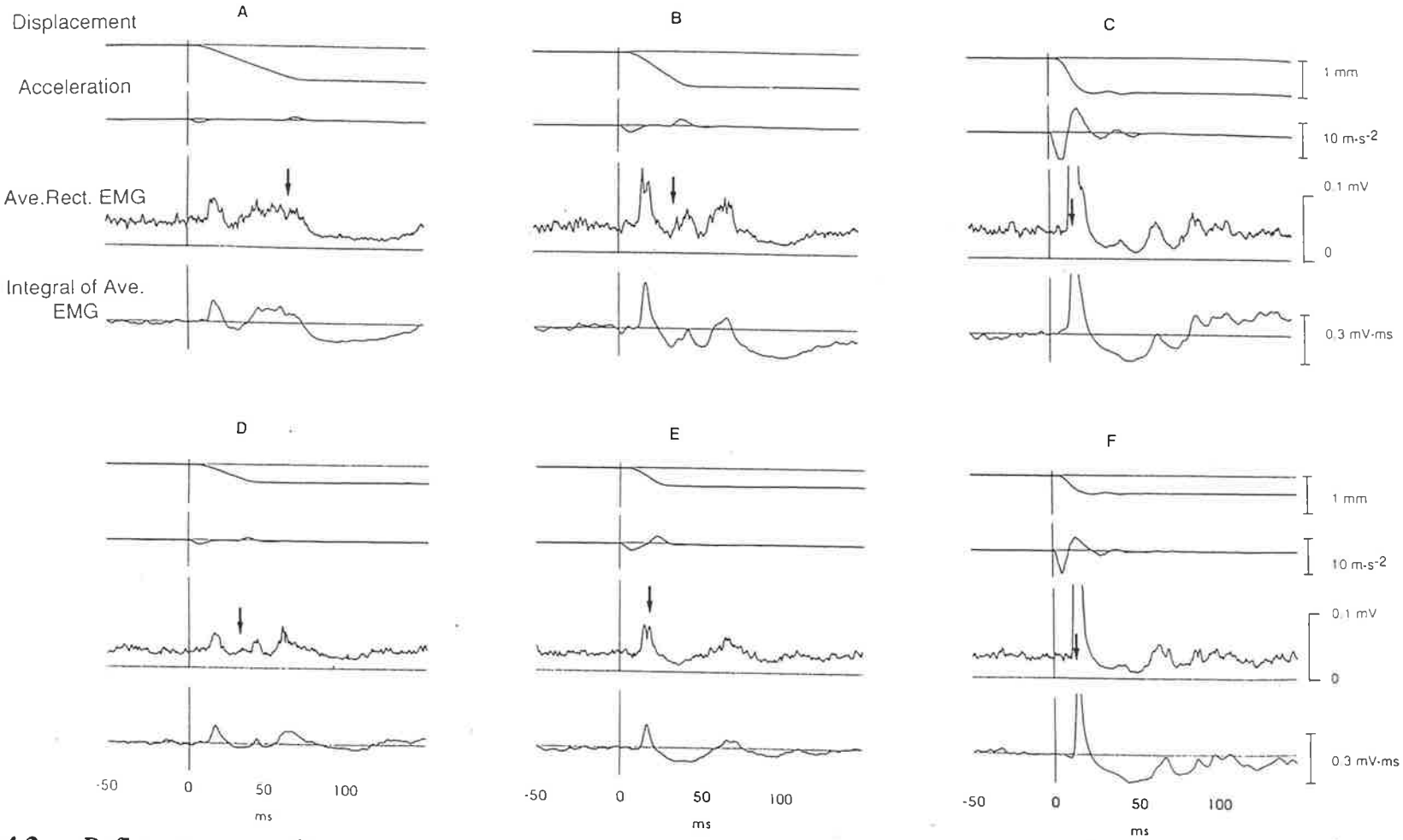

Figure 4.3 Reflex responses of human masseter to stretches of various lengths and velocities. The amplitude of the stretches was 1.0 mm in **A-C**, and 0.5 mm in **D-F**. The stretch velocities were 0.015 $m.s^{-1}$ for A and D; 0.03 $m.s^{-1}$ for **B** and **E**; and 0.1 $m.s^{-1}$ for **C** and **F**. Vertical arrows show the onset of deceleration. The disfacilitation that occurs about 10 ms after the onset of the deceleration is a major factor determining the differences in the shapes of the long-latency EMG responses to stretches of different durations.

Consider now the force produced as a result of the stimuli. The synchronous monosynaptic response evoked by the brief jaw-jerk stimuli failed to produce even a low-amplitude twitch in most subjects, e.g., Fig. 4.1A. In contrast, slower stretches evoked measurable reflex force changes in most subjects.

However, the force exerted on the jaw-bars during the slower stretch stimuli was the result of several factors. The total force changes produced by three stretch velocities in the same subject are shown in Fig. 4.4A: jaw-jerk stimuli elicited no reflex changes in force in this subject (data not shown). During the course of each stretch, there was firstly an approximately proportional increase in the force applied to the jaw-bars from the elastic properties of the jaw-closing muscles and related soft tissues. There was also a contribution to the total force from the combined inertia of the jaw-bars and the mandible. The form and time course of the inertia is shown by the acceleration records (*c.f.* Figs. 4.1B and 4.2), and is reflected in the shape of the force records in Fig. 4.4A. Although it is difficult to estimate accurately the magnitude of the inertia, the elastic plus the inertial forces produced about 10 N of the total force shown in Fig. 4.4. The inertia ceased contribute to the force at the end of the stretch. The mechanical properties of muscle can cause the force exerted by a stretched muscle to drift upwards after the stretch (Joyce, Rack & Westbury, 1969). However, the length of all three stretches shown in Fig. 4.4 was the same, and the twitch-like shape of the forces evoked by slow and rapid stretches suggest that they are mainly the consequence of reflex muscle activation. The reflexly-induced force increment in this subject was about 2 N for the slowest stretch and 5 N for the fastest.

The inverse relationship between stretch velocity and the reflex increase in force shown in Fig. 4.4A was observed consistently. In Fig. 4.4B, the maximal force occurring 80-110 ms after the onset of 0.5 mm stretches is plotted against the stretch velocity for four subjects. The actual increase in force resulting from the reflex in these four subjects was about 10-40% of the total increase in force.

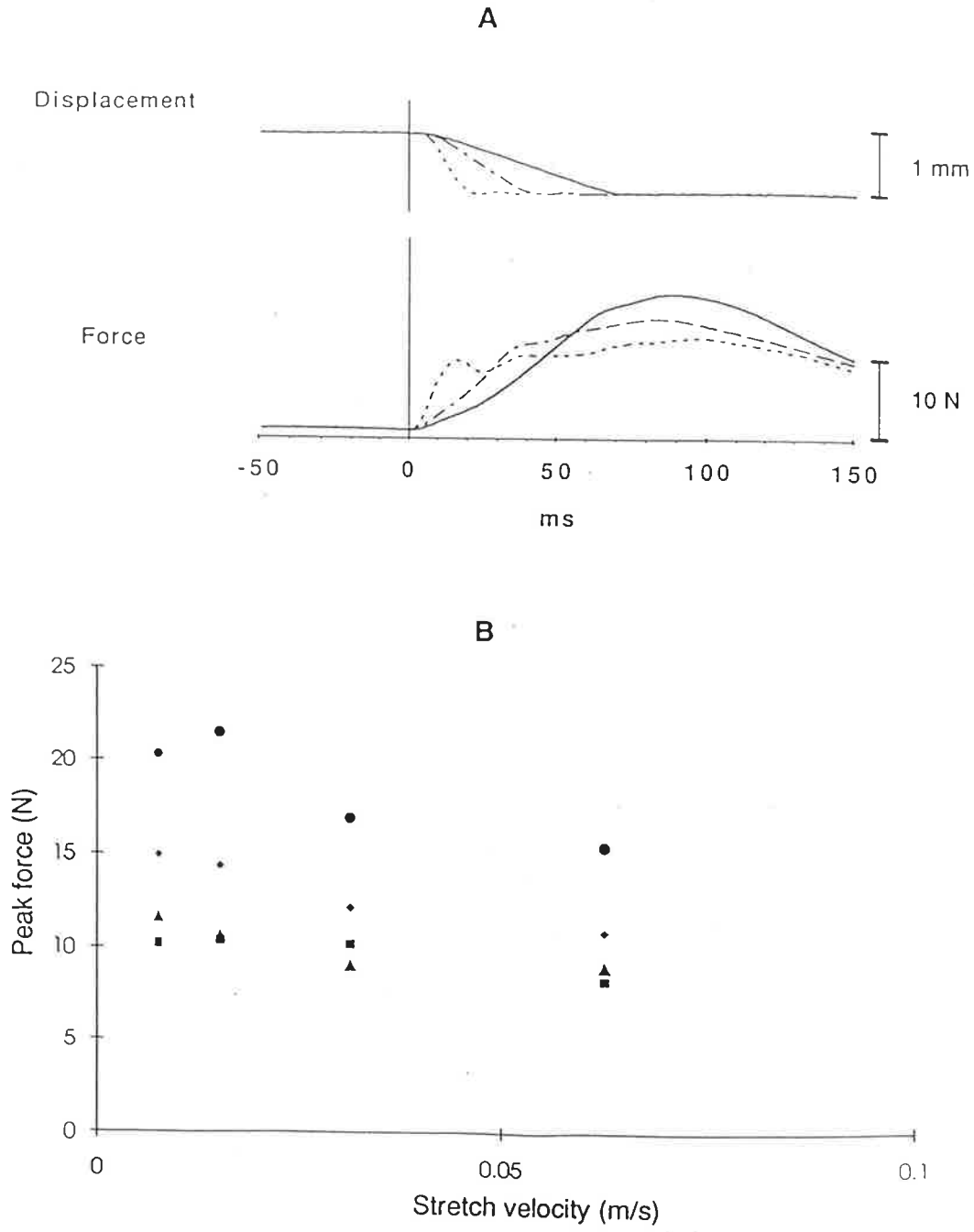

Figure 4.4 Reflexly-evoked changes in the force exerted by the jaw-closing muscles in response to various mechanical stimuli in one subject. **A**, Displacement and force records recorded in one subject during 1 mm stretches at different velocities. **B**, The peak force exerted on the jaw-bar by 4 subjects between 80 and 120 ms after the onset of 0.5 mm stretches at various stretch velocities. Slower stretches were associated with larger increases in reflex biting force.

The amplitude of the short-latency EMG response increased in proportion to the stretch velocity: in contrast, the reflexly-elicited increase force decreased as the stretch velocity increased. Hence it is clear that the reflex increases in total jaw-closing force induced by the slower stretches were the result of the long-latency reflex response evoked by these stimuli.

Reflexly-induced decreases in force were regularly observed when the jaw-closing muscles were unloaded by moving the bar 0.5 mm upwards during an isometric bite. In Fig. 4.2, for example, the force on the jaw-bars initially fell about 3.2 N when the stretch of the elastic element in the muscle was reduced, then another 4.3 N as the result of a late-onset reflex decrease in muscle activity.

4.4 DISCUSSION

It has been argued that the feedback control of muscle length in the intrinsic hand muscles is regulated principally by long-latency reflexes, with little contribution from the monosynaptic response (Matthews, 1991). However, it has been claimed that the control of the masticatory system is different, in that it is effected principally by the monosynaptic pathway. This claim is based mainly on the earlier observations that the only reflex response to stretch of the human jaw-closing muscles seen in most studies is the segmental jaw-jerk reflex (e.g., Lamarre & Lund, 1975; Cooker *et al.*, 1980; and Goodwin *et al.*, 1978). Marsden *et al.* (1976) reported that the latency of the first excitatory response to stretch occurs at 12-14 ms in masseter. This latency is too short to correspond with the long-latency response found in the present experiments. It is likely that the short-latency reflex was delayed in these records, perhaps because of the difficulty they reported in obtaining "reasonable rates of stretch" of the jaw-closing muscles.

4.4.1 Reflex Pathways

It is clear from the present study that the general pattern of reflex responses to stretch in the human masseter is similar to that in most spinal systems; that is, a burst of excitation at short latency followed at longer latency by a second phase of excitation. The rapidity of onset of the short-latency response indicates that it is at least in part monosynaptic (Lamarre & Lund, 1975). The origin of the longer-latency response has been the subject of intense debate (e.g., Wiesendanger & Miles, 1982; Matthews, 1991), but recent observations on subject with the rare Klippel-Feil syndrome confirm that the long-latency response of at least the intrinsic hand muscles is the output of a polysynaptic pathway that traverses the motor cortex (Matthews, Farmer & Ingram, 1990). Is then, the long-latency excitatory response to stretch in the masseter transcortical? It begins at about 35 ms in the integral of the average of the unrectified EMG (e.g., Figs. 4.1B, 4.3). However, the minimal time for a signal to travel from the muscle to the cortex and back

is substantially shorter than this. Non-painful trigeminal stimuli evoke a potential over the sensory cortex beginning about 8 ms (Findler & Feinsod, 1982), and the efferent conduction time from the motor cortex to the masseter is about 6 ms (Cruccu, Berardelli, Inghilleri & Manfredi, 1989), giving a minimal transcortical loop time of about 15 ms. It may be assumed that some temporal summation will be required to activate this polysynaptic pathway. If, however, the long-latency response is the output of a transcortical loop, this summation time would need to be about 20 ms, which seems excessive.

Brodin *et al.* (1993) have recently reported that pressure on an incisor tooth evokes a long-latency excitatory reflex in the human masseter. However, two lines of evidence indicate that this response is distinct from the long-latency response to muscle stretch. Firstly, the reflex response to pressure begins about 50 ms after the onset of the stimulus, which is significantly later than the long-latency response to stretch; and secondly, the long-latency response to stretch was not materially altered by local anaesthesia of the teeth, whereas this abolished the pressure-evoked response. At the present time, therefore, the pathway traversed by the long-latency response to stretch in the jaw muscles remains unknown. Why should the well-developed long-latency response seen in the present study have eluded most earlier investigators? The answer lies in the stimulus parameters used in the various studies. The present study confirmed that very brief stimuli, similar to the jaw jerks produced by a tendon hammer, evoke principally short-latency excitation. In Fig. 4.3C and F, for example, the dominant response in the EMG to brief stretches of 0.5 and 1 mm was the monosynaptic excitation. Longer stretches evoked both the short- and the long- latency excitation. Our data show clearly that the pattern of excitation is critically dependent on the duration of the stimulus. The long-latency excitation evoked by stimuli of less than about 50 ms duration is segmented by a trough that is the result of disfacilitation following the decrease in the stretch velocity (Fig. 4.3). Hence, the burst of excitation that is evoked by very brief stretches is terminated abruptly by disfacilitation. Moreover, after discharging synchronously, the

membrane potentials of many masseter motoneurons will be relatively far from their firing thresholds and thus will not be discharged by an excitatory input that arrives at longer latency (Miles *et al.*, 1989).

The extraordinary sensitivity of the monosynaptic reflex to minute vibrations of the jaw-bars may also have contributed to the absence of a long-latency response in the earlier studies. In the present experiments, the presence of vibration was revealed only by the accelerometer on the jaw-bar, and its abolition required careful tuning of the control circuitry (Miles *et al.*, 1993). If the stretches in the earlier studies induced even low-amplitude vibration of the jaw bars, they would produce a prominent monosynaptic response with no longer-latency components like that shown in Fig. 4.1A. Thus, the absence of a long-latency reflex in the records of earlier investigators is almost certainly the result of the parameters of their stretch stimuli.

It is interesting that disfacilitation has such a powerful effect on the motoneuronal activity. Fig. 4.2 shows that unloading the masseter by moving it 0.5 mm upward during an isometric bite evokes a reflex reduction in the amplitude of the average of the rectified masseter EMG to less than 20% of its pre-stimulus level. This indicates that the Ia afferents contribute substantially to the net drive to motoneurons during isometric biting, i.e., that fusimotor drive is high (Appenteng, Morimoto & Taylor, 1980). Miles & Wilkinson (1982) pointed out that powerful, rapidly-acting disfacilitation is particularly important in the jaw-closing muscles, as it can reduce the risk of the teeth crashing together during a forceful bite when the resistance to closing is suddenly and unexpectedly withdrawn. However, the major mechanism that prevents the teeth from crashing together when unloaded during a powerful isometric bite is co-activation of the antagonist muscles (Miles & Madigan, 1983).

4.4.2 Relationship of Force Changes to Reflex Responses

Cooker *et al.* (1980), who were unable to demonstrate a long-latency response in the masseter, concluded that the reflex increase in force evoked by loading the human masseter during an isotonic bite or by stretching the muscle, was the result of the monosynaptic response. In the present study, I found that, although the jaw-jerk stimuli evoke a high-amplitude monosynaptic reflex in the EMG, little or no force is generated by this electrical response in most subjects. In the jaw-closing system, as in other muscles (Matthews, 1991), slower stretches that evoke a weaker monosynaptic response but a less-synchronous, long-latency EMG response do result in the generation of a significant force response (e.g., Fig. 4.4A). The magnitude of the reflexly-evoked force is as much as 40% of the total resistance to stretch: however, the greater part of the total resistance to stretch was due to the elastic properties of the muscles and other soft-tissues. It seems paradoxical that the prominent, synchronous, monosynaptic reflex does not generate much force in the jaw-closing muscles. There are probably a number of reasons for this. First, the tightly synchronised discharge of motor units during the monosynaptic response produces a compound action potential in the surface EMG whose amplitude may lead the observer to overestimate of the number of motor units that are involved, in comparison with the broader peak that results from the asynchronous discharge in the long-latency reflex. Second, the effect of the Ia afferent volley is to bring forward the times at which some motoneurons discharge. However, as shown in the integral of the average of the unrectified EMG (which is essentially a representation of the combined PSTHs of the active motoneurons), the effect of the stretch-evoked Ia volley is to bring some motoneurons to.threshold earlier than would otherwise be the case (Miles *et al.*, 1989). This would normally produce a twitch. Then, after discharging at monosynaptic latency, these motoneurons are silent for a further interspike interval (the silent period), which is manifest in the integral as a depression below the baseline. This period of decreased motor unit activity will produce a decrease in total muscle force immediately after the twitch, thus tending to offset the effect of the twitch. Third, during the moderate

masseter contraction in the present experiments, most motor units that are active are already discharging at frequencies at which their contractions are fully fused (Nordstrom, Miles & Veale, 1989). Hence, the effect of a brief Ia volley may be only to bring into synchrony the action potentials of the already-active motoneurons, which would not result in the production of much additional force. This is somewhat analogous to the observation that no additional force is produced during a maximal voluntary contraction when a single, supramaximal shock is given to the muscle's motor nerve (e.g., McKenzie & Gandevia, 1991). Moreover, Appenteng, O'Donovan, Somjen, Stephens & Taylor (1978) have shown that, unlike most limb muscles, the masseter Ia afferents innervate principally low-threshold motoneurons: hence the probability that a stretch will recruit additional motoneurons is low. The probability of recruitment is also diminished by the fact that, in the masseter, individual Ia fibres diverge to excite only about 10% of homonymous motoneurons, compared with 80-100% in limb muscles. It would then be necessary to argue that the additional force that is produced by the long-latency reflex in masseter is due primarily to the asynchronous recruitment of additional motoneurons by the cortico-trigeminal pathway.

In functional terms, the stretch reflexes described in the present experiments may appear rather artificial, since the only stretches that are applied to the modern human masseter in the course of normal activities are the result of the mass of the mandible and the action of gravity, when one is walking or running. The significance of these reflexes may be greater to other species, including primates, when they carry heavy items of prey, or their offspring, in their mouths as they run and jump. More importantly, however, the present experiments illustrate the operation of fundamental reflex pathways in the masticatory system.

CHAPTER 5

A NEW APPROACH TO THE ESTIMATION OF POST-SYNAPTIC POTENTIALS IN HUMAN MOTONEURONS

5.1 INTRODUCTION

There is a vast literature on the measurement of stimulus-evoked post-synaptic potentials (PSPs) in the motoneurons of reduced animal preparations, dating from the first intracellular recordings of Brock, Coombs & Eccles (1952). Clearly, however, direct intracellular recordings cannot be made in awake humans who are performing some prescribed motor task. Thus, attempts to estimate the amplitude of compound PSPs evoked by various stimuli in human motoneurons have necessarily been indirect (Ashby & Zilm, 1982; Miles, Türker & Le, 1989). The question may be asked: why is it necessary to measure PSPs in human motoneurons at all, when this is more directly accomplished in animals? The answer is that the conditions that prevail in anaesthetised animals do not necessarily prevail in awake, behaving humans. It has been also shown that individual motoneurons that innervate a single muscle may respond differently to a given stimulus (Garnett & Stephens, 1980) , and the stimulus effect in low-threshold motoneurons may be opposite to that in high-threshold motoneurons (Datta & Stephens, 1981).

This chapter describes a new method for estimating stimulus-evoked PSPs in individual human motoneurons.

5.2 METHODS AND RESULTS

5.2.1 Theoretical Analysis of the Threshold Crossing Model

Before the method is described, it must be noted that it rests upon the same assumptions as those of Ashby & Zilm (1982), and Miles *et al.* (1989a). These are:

(i) that the membrane potential of a regularly-discharging motoneuron follows a linear trajectory from the trough of the after-hyperpolarisation to the firing threshold during each interspike interval (ISI), as shown by the dotted lines in Fig. 5.1;

(ii) that changes in firing rate within the relatively narrow range of frequencies over which a motoneuron will fire during voluntary contractions are the result of changes in the slope of the trajectory, with the peak of the after-hyperpolarisation remaining constant over this range; and

(iii) that compound PSPs sum linearly with the cell membrane potential.

Consider first the model resulting from these assumptions. The membrane potential of a motoneuron firing at a constant rate in the absence of noise can be represented as a continuous sawtooth-shaped waveform: when it reaches its firing threshold (FT), an action potential is discharged. The membrane potential is then reset to the peak of the afterhyperpolarisation (AHP) and the cycle repeats. The shape of the membrane potential in the absence of stimulus-evoked PSPs is shown by the dotted lines in Fig. 5.1B, C and D.

Consider now the effect of a stimulus that evokes the large, prolonged, compound excitatory post-synaptic potential (EPSP), shown in Fig. 5.1. This EPSP will be summed with the sawtooth membrane potential. Figs. B, C and D show the result of this summation when the EPSP occurs at different times relative to the last discharge of the motoneuron. It can be seen that, depending on the timing of the EPSP relative to the last action potential, the EPSP can cause the motoneuron to discharge earlier than it would

have otherwise, i.e., the duration of the ISI in which the EPSP occurred will be reduced by the amount shown by the arrows in B, C and D. A compound IPSP which took the membrane potential further from the FT would lengthen the ISI in which it occurred.

This relationship between the PSP profile and the resulting ISIs can be described mathematically. For simplicity, the FT is given the value of 0, and the minimal value of the membrane potential (i.e., the peak after-hyperpolarisation) the value -1. Let us also define a compound PSP evoked by the stimulus as the arbitrary function $E(t)$. To find the time of the discharge (t_d), one of the possible basic membrane potential trajectories $T(t)$, which are shown by dashed lines, is summed with the function $E(t)$. The linearly-changing trajectory of the membrane potential during the ISI is described by the following:

$$T(t) = a\,t + Const \tag{5.1}$$

where a is the firing frequency and $i = 1 / a$ is the interspike interval. For $t > 0$ the membrane trajectory $V(t)$ is found by summing one of the basic trajectories with the EPSP:

$$V(t) = T(t) + E(t) = E(t) + a\,t + Const \tag{5.2}$$

At the time of discharge (t_d), $V(t)$ reaches 0. Thus:

$$0 = V(t_d) = E(t_d) + a\,t_d + Const \tag{5.3}$$

or:

$$-E(t_d) = a\,t_d + Const \tag{5.4}$$

In the absence of the PSP, the same trajectory would have brought the neurone to discharge at a different time T_d.

$$0 = T(T_d) = aT_d + Const \quad (5.5)$$

Subtracting (5.4) from (5.5):

$$E(t_d) = aT_d + Const - at_d - Const = a(T_d - t_d) = (T_d - t_d)/i = C(t_d) \quad (5.6)$$

The expression $T_d - t_d$ is the change in the duration of the ISI (which will be positive for EPSPs and negative for IPSPs - shown by arrows in Fig. 5.1.

Thus equation (5.6) gives the relationship between the function of the *relative* change of interspike interval $C(t_d) = (T_d - t_d)/i$ and the function $E(t_d)$, which designates the compound PSP. If we now define the function of the expected ISI normalised to their prestimulus value i by $I(t)$ (Awiszus, 1988), we find that

$$I(t) = 1 - C(t) = 1 - E(t) \quad (5.7)$$

Both $I(t)$ and $C(t)$ are defined for times t_d at which the neurone can discharge, but note that no discharges can occur in the period shown by the dotted line in Fig. 5.1E. Thus, this model predicts that the function of relative change in the duration of the ISI after the stimulus gives an estimate of the profile of the compound PSP (lower trace in Fig. 5.1E).

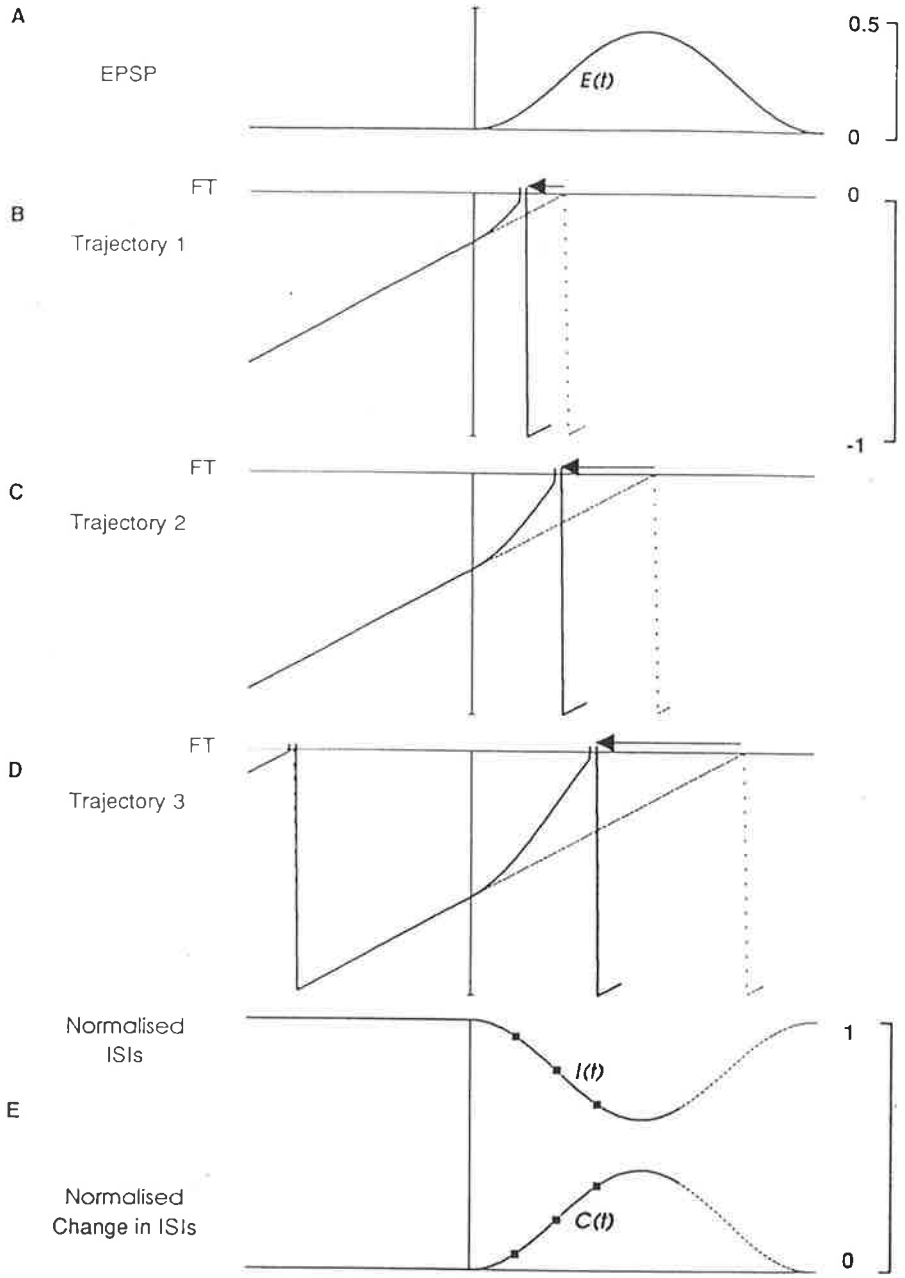

Figure 5.1 The model underlying the new analysis. The basic model is that the membrane potential of a motoneuron firing with a constant ISI in the absence of noise is described by a sawtooth waveform, as shown by the dotted lines in **B**, **C** and **D**. The membrane potential increases linearly from a notional value of -1 to the firing threshold (FT), notionally 0, at which time it discharges, and the cycle repeats. **A** shows a notional compound EPSP (same scale as **B-D**). This EPSP is presented at different times with respect to the preceding action potential in **B**, **C** and **D**, and is summed with the tonically discharging membrane potential to give the solid lines. The EPSP then brings the membrane potential to its FT earlier than otherwise, by the amount shown by the horizontal arrow in **B**, **C** and **D**. (Note that the full action potential is not shown.) In the upper trace in **E**, each of the shortened ISIs in an infinite series has been normalised to the mean pre-stimulus ISI [i.e., the function $I(t)$ in equation (5.7)], and is plotted against the time that the spike occurred. The lower trace in **E** shows the relative *change* in duration of ISIs [i.e., the function $C(t)$ in equation (5.6)], plotted against the time at which the spike occurred. It is shown in equation (5.6) that $C(t)$ is equivalent to $E(t)$.

5.2.2 Method for Reconstructing the PSP Profile from Experimental Data

The method described for estimating the profile of a compound PSP evoked by a stimulus is based on a plot of the ISI duration against the time of occurrence of each spike. In order to get a useful plot, the responses during a number of trials must be recorded. This inevitably results in plots in which the data points are scattered, e.g., Fig. 5.2. Several methods could be used to obtain the time-varying mean of these data points, which gives the profile of the PSP. However, the calculation of the mean must recognise that the data points are not uniformly distributed around the mean value at any point along the horizontal axis. For example in Fig. 5.2, whenever the unit discharges at time 0 in any trial, the next discharge can *only* occur on a straight line with a slope of +1, projecting upwards from (0,0) on the ISI plot in Fig. 5.2. Thus to find the mean ISI following all of the discharges occurring at time 0, the ISIs lying along this line must be averaged. The mean ISI of discharges occurring 10 ms after the stimulus must lie on a parallel straight line projecting from (+10,0) and so on. Thus, the average of the ISIs at each time point must be calculated by averaging the values that lie along lines with slope of +1.

This average can be calculated as follows. Let N be the number of all discharges of the motoneuron within a certain time (±T ms) of the stimulus. Let D_i be the i -th discharge time, relative to the stimulus, with all the discharge times arranged in ascending order (i running from 1 to N). Let I_i be the values of interspike intervals following each discharge. Then the averaged values for interspike intervals (A_i) can be estimated by averaging all interspike intervals with the indices within the interval $i \pm k$:

$$A_i = \left(\sum_{j=i-k}^{i+k} I_j \right) / (2k+1) \qquad (5.8)$$

This value will correspond to the discharge time D_i; therefore the discharge time of the next discharge (T_i) should be estimated as this discharge time plus the averaged interspike interval value A_i :

$$T_i = A_i + D_i \tag{5.9}$$

Plotting A_i against T_i gives the time-varying mean for the ISI plots in Fig. 5.2, and for the normalised means in the lowermost plots of Fig. 5.2. The only parameter for this method is the value of k, which was 4. Thus the running average was calculated as the mean of $2k + 1 = 9$ ISI values. Simple averaging was used in the present report, but it would be easy to modify formula (5.8) and to use weighted averaging of any sort, which will smooth the estimated profile without affecting the resolution.

One feature of this method is that each point on the time-varying mean is the average of the same number of events (motoneuron discharges). This may be superior to averages based on a fixed time window in which the number of data points averaged will vary, particularly during the peaks or the silent period of the PSTHs. The other feature of the method is that the estimate is based on the interspike intervals following the discharge. A similar procedure could be applied to the intervals that precede the discharges. This, however, may introduce an error in the profile estimate because, as pointed out by Awiszus, Feistner & Schäfer (1991), the secondary events result in modulation not of the ISI values, but of the density of dots on the ISI plot. This uneven density may result in a deviation of the time-varying average from the PSP profile. The character of these density variations follows a simple relationship. If Θ is the time of discharge of a spike, and τ is the duration of the following ISI, this event will appear as a dot with the coordinates $(\Theta + \tau, \tau)$, i.e., it will be found on the line $y = \Theta + t$. Therefore, if Θ_1 corresponds to the peak of the PSTH, the dots will be densely positioned around the line $y = \Theta_1 + t$. If Θ_2 corresponds to the silent period, there will be no dots along the line $y = \Theta_2 + t$.

The averaging procedure described here recognises this relationship and avoids mistakes that may be associated with it.

5.2.3 Application of the Method to Experimental Data

An example of the application of this analysis to some experimental data is given in Fig. 5.2. A fine-wire electrode was placed into the masseter muscle of a human volunteer. The action potentials of a single motor unit were discriminated with a computer-based waveform discriminator (SPS-8701) while the subject bit isometrically on bite bars. To justify the application of this method to experimental data, it is necessary for the subject to run the unit as regularly as possible at the target frequency with the help of auditory and visual feedback of the unit's activity. For the data shown in Fig. 5.2, the subject controlled the unit first at a mean rate of 13 Hz (upper records) and then at 10 Hz (lower records). The stimuli during both runs were controlled downward displacements of the mandible of 1 mm amplitude and 32 ms duration. The unit's responses are shown as a peri-stimulus time histogram (PSTH) and its cumulative sum (CUSUM) at each pre-stimulus firing rate. The PSTHs and the CUSUMs show that this stimulus evoked a qualitatively similar pattern of response in the unit at both pre-stimulus firing rates. The first response was excitation at a latency of 10 ms. (Note that all latencies are measured from the onset of the displacement). This increased probability of firing, manifest as a peak in the PSTH and the CUSUM, was followed by a silent period of about 25 ms. A second peak indicates a second phase of excitation beginning at about 55 ms.

The plots of ISI against the time of occurrence of the spikes show that the stimulus evoked a sharp decrease in ISI duration below the mean pre-stimulus ISI values beginning at 10 ms, at both pre-stimulus firing rates. This decrease in ISI duration is the result of a shifting forward in time of the spikes that, in the absence of a stimulus, would have discharged during the silent period (Miles *et al.*, 1989a). The ISI plots also show a second prolonged phase of decreased ISI duration beginning at about 55 ms, after which the ISI duration returns to the pre-stimulus value in both records. This corresponds to the long-latency component of the masseter stretch reflex seen in the surface EMG (see Chapter 4 and Poliakov & Miles, 1994).

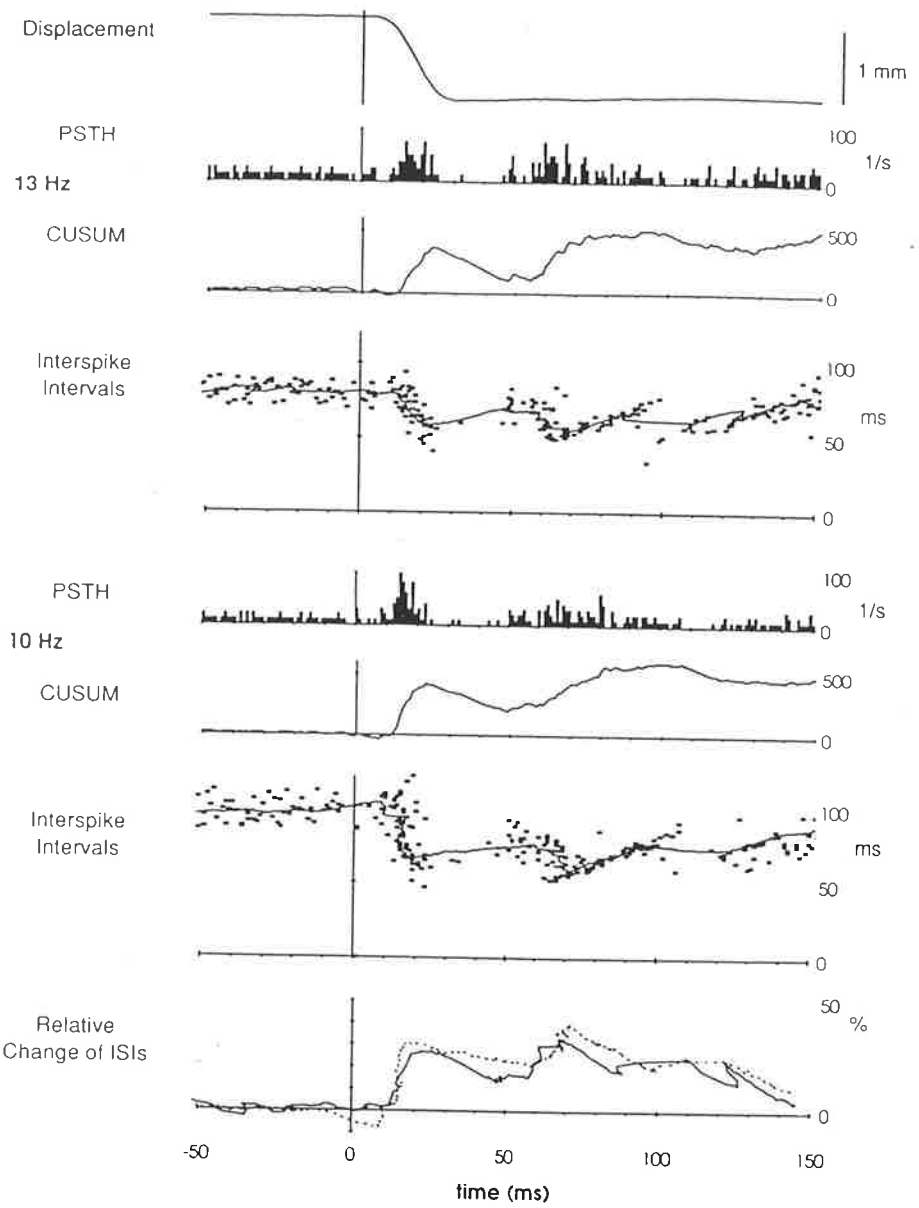

Figure 5.2 Reflex responses of a single masseter motor unit to stretch, and the estimates of the underlying compound PSP evoked in the parent motoneuron at pre-stimulus firing rates of 13 Hz (upper records, and 10 Hz (lower records), derived by the method described. The uppermost record shows the downward displacement of the mandible (1 mm amplitude, 32 ms duration) used to elicit a stretch reflex in the motor unit. Eighty stimuli were given when the unit was firing at 13 Hz, and 100 when it was firing at 10 Hz. The unit's reflex response at each firing rate is shown by the PSTH (binwidth 1 ms, expressed as probability) and its CUSUM, showing that the stretch evoked a biphasic excitatory response. Below each CUSUM, the duration of the ISI preceding each spike is plotted against the time of occurrence of the spike at that firing rate, with the time-varying mean ISI superimposed as a solid line. The lowermost record shows the relative change of the time-varying mean ISIs at both pre-stimulus firing rates; i.e., the change expressed as a percentage of the mean pre-stimulus ISI at 13 Hz (solid line) and at 10 Hz (dotted line). These lines show the shape of the compound EPSP evoked by the stimulus that would result in the pattern of discharges at the two pre-stimulus firing rates in the records above. As one would predict, the shape of the stimulus-evoked EPSP is essentially the same at the two firing rates.

The difference between the duration of the ISI at each time point and the mean pre-stimulus ISI was then calculated. These values were then normalised to the mean pre-stimulus ISIs. The time-varying means of both ISI plots were determined using formula (5.8) in section 5.2.2, and are plotted as lines on the ISI plots. Finally, the mean *relative* change in ISI at 13 Hz was plotted as a solid line in the lowermost record, and the mean relative change in ISI at 10 Hz as the dotted line. The ragged profile of segments of the averages is the result of the averaging procedure (see section 5.2.2): these irregularities could be smoothed if desired.

These lines in the lowermost records in Fig. 5.2 therefore represent the shape and time-course of the compound EPSP evoked in the motoneuron at two different firing rates (equation (5.6) in section 5.2.1). The amplitude, time-course and shape of the two biphasic compound EPSPs are similar, as one would predict for the same input. An important difference between the shape of the EPSPs and the shapes of the corresponding CUSUMs is that the CUSUMs remain elevated at 150 ms, whereas the EPSPs return to their pre-stimulus values.

In this laboratory, records such as those in Fig. 5.2 are derived from a file generated by a special-purpose computer program to produce dot raster plots. This is imported into a general-purpose spreadsheet (Excel®), and the data points are then re-organised by a "macro" to produce these plots (see Appendix A). The use of the spreadsheet's inbuilt capabilities to manipulate and plot the data in this way has been found to be a powerful and flexible tool for executing these analyses.

5.3 DISCUSSION

It is clearly desirable to make quantitative measurements of the relationship between inputs and outputs in studies of reflex function in man. Chapter 3 described a new method to achieve this for the whole muscle, using surface electromyography (see also Poliakov & Miles, 1992). However, the response of the whole muscle is the sum of the responses of its individual motor units, and different units can respond differently to the same stimulus (Garnett & Stephens, 1980; Datta & Stephens, 1981). Thus, if one wishes to determine the nature of synaptic events in human motoneurons, it is necessary to examine reflexly-evoked responses at the level of single motor units.

Several methods have been described for estimating PSPs on the basis of stimulus-induced changes in the patterns of firing rate of motoneurons. Knox (1974) proposed that the time-course of a stimulus-evoked peak in the PSTH of a motoneuron is described by the derivative of the compound EPSP that caused it. However, there are several practical limitations in using the PSTH to infer the shape of the underlying EPSP. Firstly, the method is applicable only to the PSTH peak resulting from the *first* post-stimulus spikes, since the shape of subsequent peaks and troughs will be modulated by the autocorrelogram of the motor unit. Secondly, low frequencies are poorly represented in the derivative of a signal, which makes this method unsuitable for estimating slow EPSPs.

A different approach to the estimation of the PSP shape in a motoneuron that is firing tonically was developed by Miles *et al.* (1989a, b). This approach requires that stimuli be given at precisely timed intervals following the preceding spike. A compound EPSP that occurs as the membrane potential is approaching the FT will then advance the timing of the next action potential by an amount that depends upon the waveform of the EPSP. An IPSP will delay the next action potential. By spacing the stimuli throughout the duration of an ISI in successive trials, one obtains an estimate of the shape and amplitude of the PSP. This method has several advantages over the PSTH approach. Firstly, it is a direct

empirical measurement. Secondly, because it depends on changes in the *duration* of ISIs rather than peaks in PSTHs, it can be used to estimate the shape of complex duration EPSPs and/or IPSPs, i.e., the PSP estimates are not distorted by the unit's autocorrelogram as they are in methods based on the PSTH. In practice, however, this method is less satisfactory when applied to long-latency PSPs, since the shape of the later part of the PSP is seen only when each trial in the raster is re-aligned against the first spike to occur after the stimulus, instead of against the stimulus itself. This procedure doubles the noise-induced scatter of data points in the second ISI.

The present method of plotting ISIs against time was first used by Awiszus *et al.* (1991) to avoid the autocorrelation function that is inherent in the PSTH and its CUSUM, thus enabling reflexly-evoked activity (i.e., true excitatory and inhibitory responses) beyond the first post-stimulus discharge to be identified unambiguously. I have now shown that this representation can be used to estimate the shape of the PSP evoked by a stimulus. The major advantages of the present over earlier methods for estimating the PSP are that it displays the time course of both the early and the later components of complex PSPs, and that the scatter of the data points does not increase with time after the stimulus. Consequently, it is particularly suitable for estimating the shape of long-duration PSPs. A further advantage of the new method is that random fluctuations in the baseline will result in a fluctuation of the density of data points, but will not distort the estimate of the shape of the PSP.

Consider now the validity of the assumptions that underlie the model. It should first be noted that the model is intended not to give a precise description of the membrane potential of a steadily-discharging motoneuron, but to give a working approximation of it. The model does not attempt to include all of the known properties of motoneurons, e.g., dependence of the FT on firing rate, plateau potentials, etc. That is, it is not intended to be a so-called analytical model of a motoneuron, based on equations derived from conceptual models of a biological process. Rather, it is an empirical model, which is

adopted not ***because*** it is correct in every detail (no model is), but ***as if*** it is correct in the sense that it gives a good approximation of the behaviour of the system tested in a given experimental paradigm (Keen & Spain, 1992). The use of such models is justified when it is not feasible to record directly the variable of interest (i.e., the membrane potential of a human motoneuron), because they have predictive ability, and because they lead to the design of new experiments that will refine the model further.

There are several lines of experimental evidence indicating that the present model does give a good approximation of the membrane potential within the narrow range of frequencies at which a human motor neurone can discharge voluntarily. For example, the model assumes that the membrane potential approaches the FT with a linear trajectory. Although not explicitly stated, this assumption is implicit in Knox's (1974) original proposal that the derivative of the PSP may be represented by the PSTH. This assumption was later supported experimentally by the observation that, at least for large EPSPs, the derivative of the PSP recorded intracellularly in cat motoneurons resembles the PSTH. However, the match was poor for smaller EPSPs in the presence of appreciable synaptic noise (Fetz & Gustafsson, 1983).

The model also assumes that the slope of the membrane potential trajectory during an ISI increases with the firing rate. For the sake of simplicity, the model assumes that the peak amplitude of the AHP is constant at different firing rates. In fact, the peak amplitude of the AHP need not be constant in the model, but the trajectory of the membrane potential should approach the FT as if it is projected linearly from a notionally constant AHP trough. One important consequence of these two assumptions is that the probability that a given stimulus-evoked EPSP will bring the motoneuronal membrane potential to threshold is independent of the firing rate of the motoneuron. This has been demonstrated for the H-reflex in the human soleus (Miles *et al.*, 1989a), and is confirmed in the present study for a biphasic stretch reflex (Fig. 5.2). It also follows from the model that the duration of inhibition resulting from a given IPSP would be less when the

pre-stimulus firing rate of the motoneuron increased. Again, this has been shown experimentally (Miles *et al.*, 1987; Miles *et al.*, 1989b).

However, any prediction arising from a model can approximate the system only as closely as the model approximates the physiological phenomena that it is intended to describe. One of the most serious simplifications of this model is the absence of synaptic noise and/or other sources or randomness in the membrane trajectory. The obvious consequence of noise is the resulting scatter of the data points, which can be dealt with by averaging. A less obvious, but more important consequence of this simplification is that, when synaptic noise is present (i.e., in experimental data), the shape of the peak evoked in the PSTH is a distorted version of the EPSP derivative. This was demonstrated when the derivative of the PSP recorded intracellularly was compared with the PSTH of the same neuron (Knox & Poppele, 1977; Kirkwood & Sears, 1978; Fetz & Gustafsson, 1983). The match was improved by combining the PSP with its derivative (Kirkwood & Sears, 1978). When the noiseless model is modified by the introduction of a Gaussian stochastic process representing synaptic noise (Kostyukov, 1978), mathematical analysis of the model becomes much more difficult, and only a numerical solution is possible. Using this, I found a good qualitative agreement between the predicted shape of the PSTH and experimental data (Polyakov, 1991). The possible distortion due to noise in the new method has not been analysed, but should not be critical for large, slow PSPs.

The present method was developed to estimate the PSPs evoked by sensory inputs in human motoneurons. As the examples show, the method will show the shape of the leading edge of EPSPs, and the trailing edge of slow EPSPs. It is less effective at showing the leading edge of sharp IPSPs because the large increases in ISIs induced by IPSPs leave gaps in the display (e.g., Fig. 5.2). The time course and trailing edge of IPSPs is well displayed.

This method can be used to examine synaptic interactions between any steadily discharging cell in the nervous system and any input, under conditions when extracellular

but not intracellular recordings are possible. Examples include extracellular recordings from central neurones in unanaesthetised animals implanted with a head-cap, or from small-diameter cell bodies or axons within the CNS. However, the interpretation of the results will depend upon how applicable this model of repetitive discharge is to the neurone in question.

CHAPTER 6

RESPONSES OF HUMAN MASSETER MOTOR UNITS TO STRETCH

6.1 INTRODUCTION

The organisation of the reflex pathways controlling the jaw muscles differs in several respects from those controlling more commonly studied limb muscles (see Chapter 1). For example, the jaw-closing muscles have many muscle spindles, but the jaw-opening muscles apparently lack them (Rowlerson, 1990; Appenteng, 1990). The jaw muscles receive no reciprocal inhibition from antagonist muscle spindles, and also lack recurrent axon collaterals and Renshaw inhibition (Shigenaga, Yoshida, Tsuru, Mitsukiro, Otani, & Cao, 1988). The proportion of homonymous motoneurons receiving an input from a single Ia afferent is much lower in the jaws (Appenteng, O'Donovan, Somjen, Stephens & Taylor, 1978; Nozaki, Iriki., & Nokamura, 1985) than in the limbs (Mendell & Henneman, 1971; Watt, Stauffer, Taylor, Reinking & Stuart, 1976). When present, unitary excitatory post-synaptic potentials (EPSPs) in jaw-elevator motoneurons from single spindle afferents are several times smaller than those in hindlimb motoneurons (Appenteng *et al.*, 1978). Until recently, it was believed that the jaw muscles lacked long-latency stretch reflexes, but we have recently shown that slow, smooth displacements of the jaws produce both short- and long-latency reflexes in the masseter surface EMG (see Chapter 4 and Poliakov & Miles, 1994).

The responses of single motor units in human masseter to muscle stretch are examined in this Chapter. This approach reveals in finer detail the pattern of inputs from muscle

spindle afferents to masseter motoneurons. It is not known, for example, whether the Ia afferents are restricted to a sub-population of the homonymous masticatory motoneuron pool, or whether there is a weak, but uniform projection to all motoneurons in the pool. A related issue is whether individual motor units contribute to both the short- and long-latency phases of the reflex as in human *flexor carpi radialis* (Calancie & Bawa, 1985a). This is of particular interest in the masseter because of the differences in spindle inputs to jaw motoneurons in animals, and also because the long-latency reflex was previously thought to be absent in the masticatory muscles. The third aim was to use the single motor unit discharge patterns to explain the pattern of forces that are evoked reflexly by stretch. Reflexly-evoked force responses are due principally to the long-latency phase of the reflex in the limbs (Rothwell, 1987), and jaw muscles (see Chapter 4 and Poliakov & Miles, 1994). This was investigated by analysing stimulus-locked changes in the durations of motor unit ISIs (Awiszus, Feistner & Schäfer, 1991 and Chapter 5), in contrast to changes in discharge probability.

6.2 METHODS

The experiments were conducted with the approval of the Committee on the Ethics of Human Experimentation at The University of Adelaide. The subjects were 7 volunteers aged 20-46, who gave informed consent and participated in 11 experiments.

6.2.1 Apparatus and Recording

Surface electrodes were placed on the skin overlying the right masseter muscle. One electrode was at the level of the lower border of the mandible, and the other 25 mm above this, close to the motor point. Preliminary experiments showed that with this placement the waveform obtained by triggering an average of the surface EMG on the spikes of a single masseter motor unit was biphasic and approximately symmetrical. This is desirable when using the integral of the average EMG to quantify the reflex responses (Chapter 3 and Poliakov & Miles, 1992). One or two intramuscular fine-wire electrodes were inserted into the muscle to detect the activity of single masseter motor units.

Subjects were seated comfortably so that they could bite isometrically with their incisor teeth on a purpose-built, jaw-muscle stretcher which is described in detail elsewhere (Chapter 2 and Miles, Poliakov & Flavel, 1993). The initial position for most stretches was with the incisor teeth about 3 mm apart. Jaw-closing force was measured by a strain gauge on the lower jaw-bar, and displacement and acceleration of the lower jaw bar were measured with a length transducer and accelerometer mounted on the apparatus. Surface EMG (bandwidth 2-500 Hz), force, displacement and acceleration were digitised on-line (1 kHz sampling rate per channel) and averaged with respect to the stimuli using a Macintosh IIci computer and custom software (LabView). Surface and intramuscular EMG, jaw-closing force and displacement were also recorded on digital tape.

6.2.2 Protocol

The action potentials of a single masseter motor unit were discriminated on-line. During each recording run, the subject was instructed to bite so that the unit discharged steadily at a firing frequency that was comfortable for the subject and consistent with good discrimination of the unit potential. This was achieved with the help of auditory and visual feedback of the firing rate. Each run consisted of 50 or 100 trials, in which the stimulus was a smooth, downward displacement of the mandible. Stimuli were given at randomly timed intervals of not less than 1.5 s. The characteristics of the ramp displacements (duration and rise-time) were specified in a special-purpose program on a personal computer. The amplitudes of the displacements were 0.5 and 1.0 mm, and durations were 8, 16, 32 and 64 ms. The actual displacement of the jaw bar followed the control signal waveform with a constant delay and minimal vibration (e.g. Figs. 6.1, 6.2, 6.4, 6.5, 6.6).

6.2.3 Data Analysis

Both the rectified and unrectified surface EMG were averaged, and the integral of the average of the unrectified surface EMG was calculated. The latter representation has some theoretical advantages over the conventionally-used average of the rectified EMG: in particular, it is linearly related to the sum of the PSTHs of single motor units (Chapter 3 and Poliakov & Miles, 1992).

Motor unit action potentials were discriminated off-line from the taped records with a computer-based waveform discriminator (SPS-8701D) which uses a template-matching algorithm. It must be stressed that only data that was discriminated with high accuracy (no false positive acceptances and less than 1% of spikes missed due to superimposition) were included in the present report. On some occasions, motor units other than the feedback motor unit were identified in records from the same or a different electrode, and their data were also analysed provided discrimination accuracy was acceptable.

Motor unit discharge times (250 μs resolution) were presented as rasters of discharges aligned to the stimulus. In this presentation of the trial-by-trial responses of the motor unit, the sequence of rows in the raster is simply ordered by the trial number (e.g. Fig. 6.1). The individual trials were also rearranged into a more informative form which is termed the *sorted raster*. In this presentation of the data (e.g. Fig. 6.1, bottom), the sequence of trials from top to bottom was rearranged. Each trial was placed so that the delay between the stimulus and the preceding spike in that trial became the Y coordinate of the plot. The sorted raster clarified the relationship between the time of the preceding discharge and the unit's response to the stimulus.

The reflex effects of the stimulus on motor unit discharge were quantified from the peristimulus time histogram (PSTH - binwidth 1 ms) and its cumulative sum (CUSUM). The CUSUM is the cumulative change in discharge probability evoked by the stimulus (Ellaway, 1978). Two response indices derived from the CUSUM were used to describe the motor unit response to the stretch. The earliest short-latency reflex response occurred 10-15 ms after the stimulus in all motor units. The short-latency response index (RI_S) was given by the maximal CUSUM value in the interval 10-20 ms after the stimulus. The maximal CUSUM value for the interval 10-100 ms after the stimulus was used as an index of the total response to stretch (RI_t). Both indices were normalised to the number of trials in the experiment, and were positive values within the ranges 0-0.5 for RI_S and 0-1.0 for RI_t.

These response indices were also analysed statistically, since the CUSUM can deviate from the baseline by chance alone. This problem has been approached in the article of Davey, Ellaway & Stein (1986), and it has been shown that this deviation can be characterised by variance (V), the function of delay from the reference point (t), number of trials (N) and mean pre-stimulus ISI (I) as follows:

$$V = (N t) / I$$

Thus, some judgment about the significance of the deviations observed can be made by comparing the CUSUM profile with a certain envelope. This, however, does not provide estimates of P values, as do standard statistical tests.

The following estimate of statistical significance of response indices is based on the considerations of Davey *et al.* (1986). The formula for standard deviation (σ) at a certain time point for the CUSUM ***normalised*** to the number of trials is:

$$\sigma = [V]^{1/2} / N = [t/(IN)]^{1/2}$$

This is an estimate of deviation possible by chance only. For example, with I = 80 ms, N = 50 trials and t = 10 ms, the standard deviation was estimated to be 0.05 and compared with RI_S. With N = 100 trials, it becomes 0.035. For RI_t these values increase 3 times, since t = 90 ms. The values for RI_S and RI_t were accepted as ***significant*** if they exceeded the predicted σ values by more than a factor of 2. If they were 2.6 times as large they were considered to be ***highly significant*** (note that for the normal distribution the interval of $\pm 2\ \sigma$ covers more than 95% of the distribution, and 2.6 σ covers more than 99%). This procedure is a conservative estimate of the statistical significance, and even more so for the longer time intervals, since the motoneuron discharges do not follow Poisson statistics (Davey *et al.*, 1986).

The σ also gives an estimate of the error expected when measuring response indices. It was found to be in good agreement with the SDs of response indices of repeated runs of 50 trials of the same motor unit under identical conditions. The value σ = 0.05 was regarded as the worst case value, and was used for estimation of the confidence limits.

The final analysis was a plot of ISI values against the peristimulus discharge time (Awiszus *et al.*, 1991). This time-varying plot gives a new insight into the mechanism underlying the reflex force changes evoked by stretch (e.g. Fig. 6.3), and can also be used to estimate features of the post-synaptic potentials evoked in the motoneuron by the stimulus (Chapter 5). The fitted line of averaged ISI values in Figs 6.1, 6.4 and 6.5 was

calculated using the method described in detail in section 5.2.2 of Chapter 5 (with the parameter $k = 4$; this correspondes to averaging of $2k+1 = 9$ ISI values).

6.3 RESULTS

6.3.1 Patterns of Reflex Responses of Individual Motor Units

Examples of the analyses used to characterise the surface EMG and motor unit responses to stretch are shown in Fig. 6.1. In this example, the stretch was a downward displacement of the mandible of 1 mm amplitude and 16 ms duration (velocity 63 $mm.s^{-1}$). This evoked both a short- and long-latency reflex response evident in the surface EMG averages. The remaining traces in Fig. 6.1 show the responses of a single masseter motor unit that was active during these trials. The raster, PSTH and CUSUM analyses are conventional, and show that the motor unit had periods of altered discharge probability that coincided with phases of the reflex seen in the surface EMG records. The short-latency response index (RI_S) in this example was 0.20, which was highly significant (see section 6.2). The overall response index (RI_t) was 0.45 (highly significant). As was commonly the case, the integral of the unrectified averaged EMG matched the unit records more closely than the average of the rectified EMG or the CUSUM of the PSTH motor unit records and surface records (see also Fig. 6.2). The rectified EMG average consistently underestimated the duration of the silent period.

The two lowermost traces in Fig. 6.1 show the two additional analyses used to examine the responses of motor units to stretch. The plot of ISIs *vs* time reveals that during the short-latency phase of the reflex (10-20 ms) there was little shortening of individual ISIs. The data points remain distributed about the mean ISI of 80 ms in this period. The ISI plot indicates that there was a more substantial shortening of ISIs during the period 50-130 ms after the stimulus. The reaction time in masseter is around 100 ms (Brodin, Türker & Miles, 1993), so activity prior to this point can be ascribed to reflex events. The long-latency phase of the reflex in this case occurred between 45 and 100 ms after the stimulus, and the maximum shortening of ISIs (to around 50 ms) occurred at 60-100 ms latency. Note that it is not evident from the PSTH and its CUSUM that the long-latency phase lasted this long.

It is important to emphasise that, while the prominent short-latency peaks in the PSTH and CUSUM indicate that the probability of discharge of the unit is increased during this period, the duration of the ISIs is decreased only very slightly. This was a very consistent observation. The explanation for this apparent anomaly becomes clear when one examines the sorted raster in which the row sequence of the conventional raster has been rearranged so that the delay between the stimulus and the preceding spike in that trial became the Y coordinate of the plot (lowermost record in Fig. 6.1). The expected time of the first discharge following the stimulus is shown by the dashed line. This presentation reveals two points about the discharges that followed the stimulus at short latencies (10-20 ms); a) they only occurred in trials in which the stimulus occurred at least 40 ms after the last spike, and b) the shortening of the ISI was relatively small. The duration of the following silent period is about 30 ms in the PSTH; therefore it can be estimated that the discharges that occurred at about monosynaptic latency were advanced on average by about half of this value, i.e., 15 ms or 20%, which is within the normal fluctuation of ISIs.

The motor unit never discharged at the short latency in trials in which the last pre-stimulus discharge occurred less than 40 ms before the stimulus; in those trials, it discharged during the long-latency phase of the reflex (50-70 ms). The unit was excited at short latency only if the last pre-stimulus discharge occurred more than 40 ms before the stimulus, and it then also responded during the long-latency phase (45-100 ms). The duration of the second ISI after the stimulus was also shortened, contributing to the large reduction in mean ISI during the long-latency phase of the reflex.

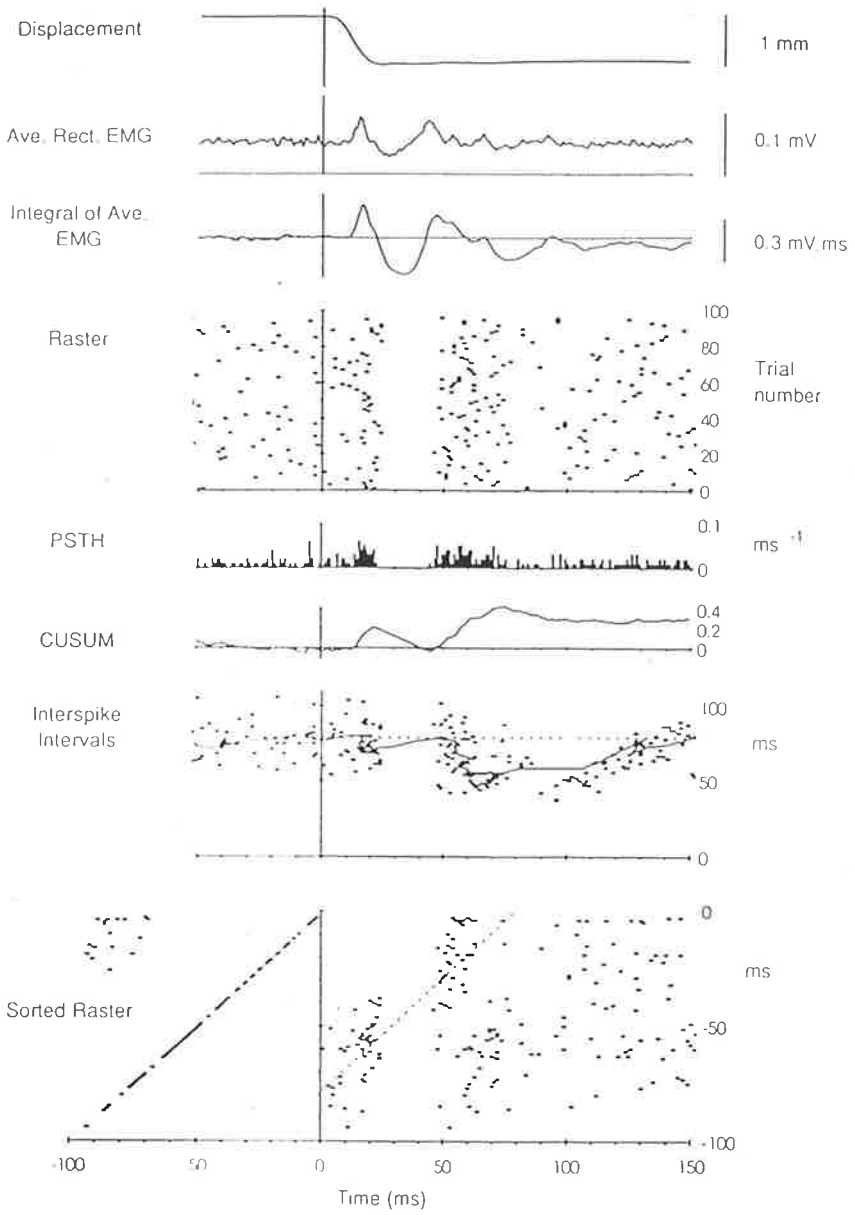

Figure 6.1 Surface EMG and single motor unit responses to randomly timed stretches of the human masseter. The upper three traces are displacement (opening downwards), the average of the rectified masseter surface EMG, and the integral of the averaged masseter surface EMG. This stimulus evoked both a short- and long-latency reflex response in the surface EMG records. The remaining records are from a single motor unit active throughout this run. The upper raster shows an enhanced spike density at short and long latencies, separated by a silent period. The PSTH and CUSUM show peaks at both short- and long-latency separated by a period of reduced discharge probability. The two lowermost records provide two additional analyses that reveal information that is not apparent in the surface EMG records or in the conventional motor unit analyses above. The ISI *vs* time plot shows that the ISIs are shortened by up to 50% throughout the long-latency phase of the reflex. The lowermost record shows the same data that is presented in the raster plot, reorganised so that each trial (row) is placed so that the delay between the stimulus and the preceding spike in that trial is the Y coordinate of the plot. This presentation shows that the phase of the reflex in which the motor unit responds in a single trial depends on the time since the preceding discharge of the motor unit.

6.3.2 Comparison with the Surface EMG Data

Fig. 6.2 shows the reflex response to stretch of the masseter in both the surface EMG and in two concurrently active motor units. The stretch was a smooth, downwards displacement of the mandible (1 mm amplitude and 16 ms duration; 63 $mm.s^{-1}$). The subject was given feedback of the activity of unit A, and was required to control its mean discharge rate at 17 Hz. The firing frequency of unit B during the experiment was about 20 Hz. The major phases of the reflex pattern in the surface EMG are a short-latency excitatory response beginning at 11 ms, followed by the depression of activity due to the silent period and the disfacilitation caused by the deceleration phase of the stretch; and finally a long-latency excitatory phase beginning at around 40 ms. These phases of the reflex were also present in the motor unit PSTHs. The integral of the averaged EMG more closely resembles the discharge of single motor units than the rectified EMG average. For example, the depression phase is barely noticeable in the averaged rectified EMG and its duration is less than in the summed PSTH. The PSTHs of the motor unit discharge also show a depression at a latency of about 70 ms, this being clearly seen in the integral of the averaged EMG, but not in the averaged rectified EMG. The pattern of responses seen in the sum of the PSTHs of the two motor units was generally in good agreement with the surface data. Note that there is a small discrepancy in the latencies estimated from the surface and motor unit data. In Fig. 6.2, the peak of the short-latency response is estimated to be 12 ms in the surface record and 15 ms in the motor unit records. This difference corresponds to the propagation delay through the muscle. This discrepancy is relatively more important in cranial muscles in general than in limb muscles, because of the very short conduction distances of the reflex arcs. A discrepancy of 3 ms is 25% of the short-latency loop time in the masseter, compared with approximately 10% in a muscle such as soleus.

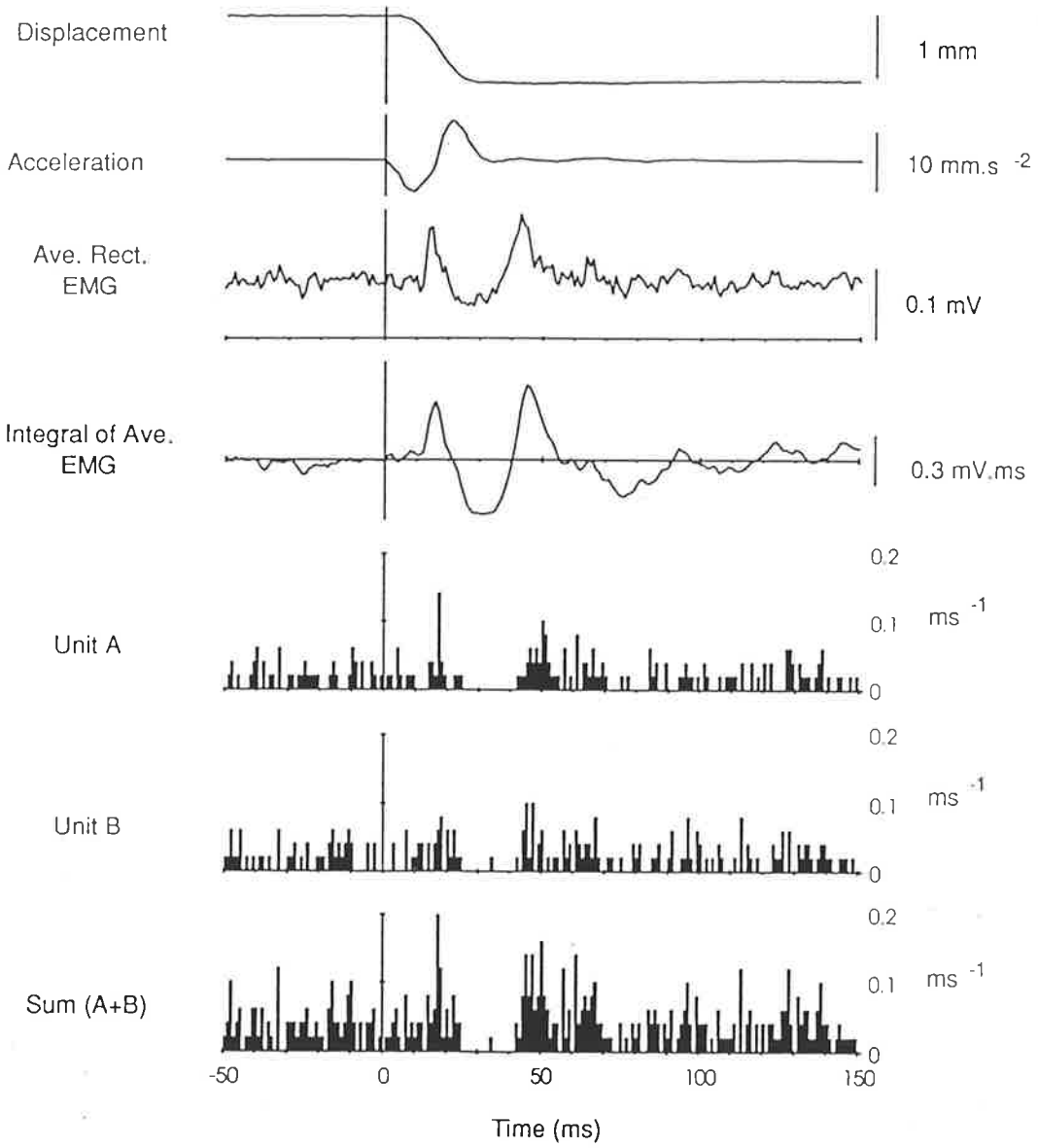

Figure 6.2 Comparison of reflex responses in two concurrently active motor units and the surface EMG reflex pattern in masseter. The uppermost traces are displacement (opening downward) and acceleration of the jaw bar. The next two traces show the reflex responses to this stretch observed in the averages of the rectified and unrectified surface EMG. In both analyses, there is a prominent short-latency peak beginning at around 11 ms, followed by a silent period, then a longer-latency excitation beginning around 40 ms. The three lowermost records are PSTHs of the responses of two concurrently active masseter motor units (A and B) to this stretch. The PSTHs of units A and B are summed in the lowermost trace. The reflex pattern in the PSTHs of the motor units is similar to that seen in the surface records.

6.3.3 Reflex Response to Slow and Brisk Stretches

The responses of a motor unit to 1 mm stretches of 8 and 64 ms duration (stretch velocities of 125 and 16 $mm.s^{-1}$, respectively) are shown in Fig. 6.3 A, B. The slow stretch (Fig. 6.3A) produced little modulation of motor unit discharge at short latency (10-20 ms), but produced a prominent peak in the CUSUM and prominent shortening of ISIs during the long latency (50-100 ms) phase. The faster stretch (Fig. 6.3B) produced a large short-latency peak in the PSTH and CUSUM, and a smaller long-latency response. The force records in Fig. 6.3A, B show that the faster stretch produced a large initial passive force, but the reflex change in force after 100 ms was larger in the slow-stretch trials. The observation that slow stretches produce a larger reflex jaw-closing force than brisk stretches (Poliakov & Miles, 1994) can be explained by the motor unit ISI records. Like Fig. 6.1, these show that the synchronous short-latency peak (Fig. 6.3B) was not accompanied by marked shortening of ISIs in this period. A comparison of the two stretches reveals a smaller shortening of ISIs during the long-latency phase following the faster stretch. That is, despite the impressive synchronous short-latency peak in the PSTH and CUSUM with the faster stretch, the modulation of motor unit ISIs was more substantial in the slow stretch trials. It is the change in ISI (or frequency) that determines the force produced by a tonically active motor unit, and this is greater with the slow stretch.

Fig. 6.3C shows the dependence of the short-latency and overall response indices on the velocity of a 1 mm stretch. Data are from 8 masseter motor units that were tested with 1 mm stretches and at least 2 different stretch velocities. The short-latency response index is larger with higher stretch velocities. The response index characterising the overall reflex response decreases at higher stretch velocities.

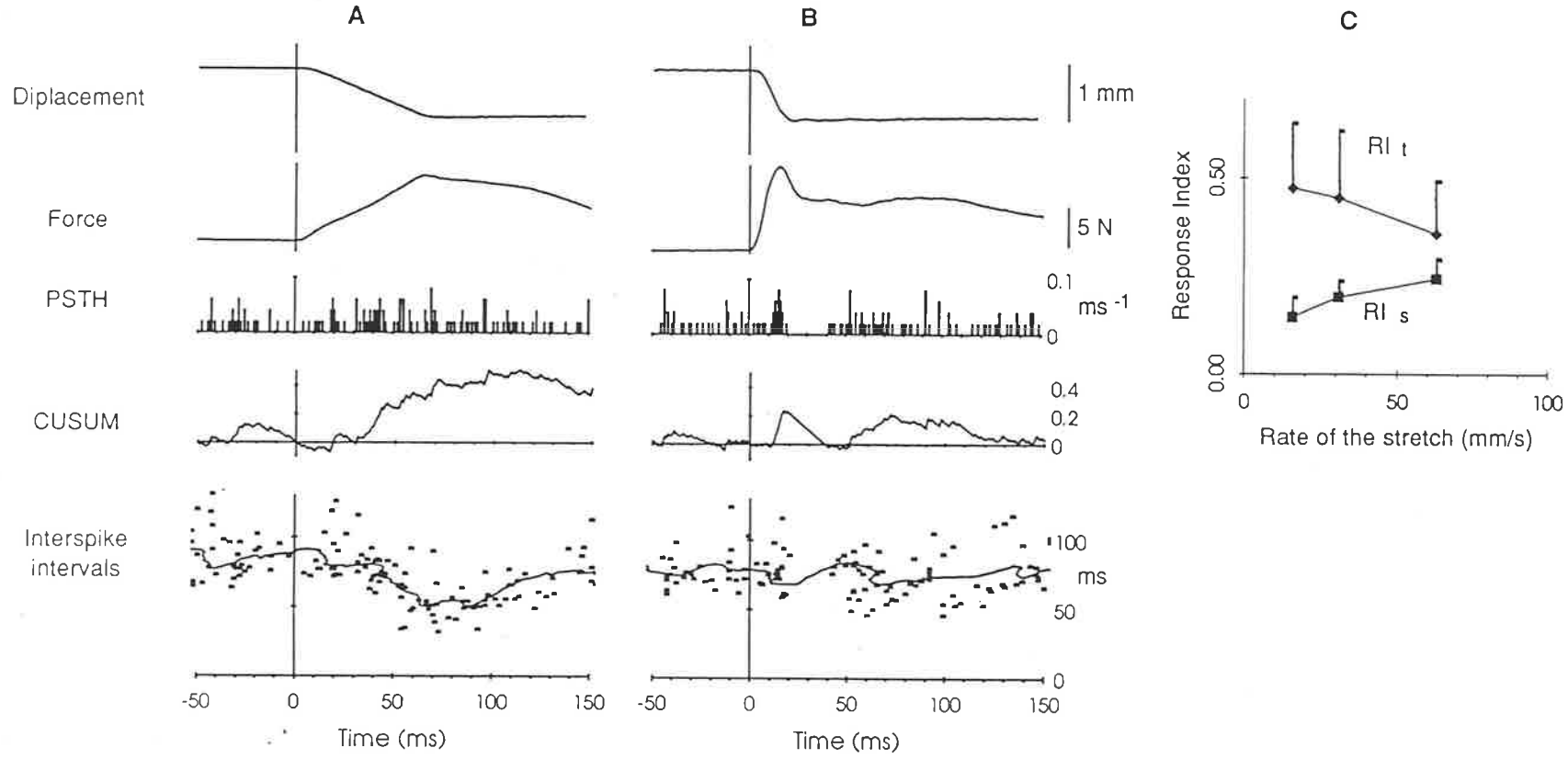

Figure 6.3 Effects of stretch velocity on motor unit reflex responses. **A**, slow stretch (1 mm at 16 $mm.s^{-1}$). Upper traces are displacement (opening downwards) and force. Below these are some of the analyses (PSTH, CUSUM, ISI plot, normalised change in ISI plot) used to characterise the reflex responses of a single motor unit to the stretch. The slow stretch evoked only a long-latency reflex response. **B**, faster stretch, (1 mm at 125 $mm.s^{-1}$), data arranged as in **A**. Note the large initial passive component to the force response, including the inertial component, after which the force falls below the level produced by the stretch in **A**. There is a prominent short-latency peak in the PSTH and CUSUM, but little shortening of the ISIs in the short-latency period. The shortening of ISIs in the long-latency phase is smaller than that elicited by the slower stretch. **C**, summary of responses for 8 motor units with 1 mm stretches that were tested with at least 2 different stretch velocities. Mean (+ SD) response indices are shown for short-latency (RI_S) and overall (RI_t) reflex responses. Short-latency responses were larger at higher stretch velocities, but the overall reflex response was larger at the slower stretch velocities.

6.3.4 Diversity of Responses in Individual Motor Units

The responses of different motor units to stretch differed markedly from one unit to another. The RI_S determined at an intermediate stretch velocity (31 $mm.s^{-1}$) ranged from 0.02 to 0.35: the distribution of these RI_S in 22 units is summarised in Fig. 6.4A. Eight motor units (35%, open bars on the histogram) did not have a significant short-latency reflex response at this stretch velocity. Four of these eight units were tested repeatedly at this stretch velocity (>320 trials), and these data were merged for analysis. Significant short-latency peaks were not demonstrated, although with this large number of trials an RI_S of about 0.03 would be significant. Eight of these twenty-two motor units were also tested with the 63 $mm.s^{-1}$ stretch, and 3 still had no significant short-latency peak. In contrast, 14 units had statistically-significant short-latency peaks with a stretch velocity of 31 $mm.s^{-1}$, and 13 units showed prominent and statistically-significant short-latency responses with a stretch velocity 16 $mm.s^{-1}$. A stretch velocity of 16 $mm.s^{-1}$ evoked a detectable short-latency reflex response in the surface EMG averages in all 7 subjects, so it seems clear that the threshold for short-latency reflex responses varies considerably in different motor units. The short-latency response of different units to the same stretch varied over at least one order of magnitude.

For the pooled data from all subjects, there was a significant positive correlation between the short-latency response index of a motor unit following brisk stretch (32 $mm.s^{-1}$ stretch velocity) and the long-latency response index with a slower stretch (16 $mm.s^{-1}$ stretch velocity) ($r^2 = 0.46$, $n = 13$, $P < 0.02$). That is, units with a larger short-latency response to brisk stretch tended to have a larger long-latency response to slow stretch. This correlation may have been influenced by subject differences in stretch responsiveness, but there are insufficient data for within-subject comparisons on this point.

Different responses were even found in units being recorded concurrently. For example, Fig. 6.4B shows the responses of two different motor units recorded from separate

electrodes during the same trials to a stretch of 0.5 mm amplitude and 16 ms duration. The mean discharge rate of unit A was 11 Hz, and its PSTH and CUSUM show a significant excitatory peak at a short latency ($RI_S = 0.22$). In contrast, the short-latency peak in the CUSUM of unit B, which had a mean discharge rate of 14 Hz during the run, was not significant ($RI_S = 0.02$). In general, units with higher discharge rates at a given force level had lower recruitment thresholds. In this case, the unit with the lower recruitment threshold had no detectable short-latency reflex response to stretch, while the higher threshold unit had a large short-latency response.

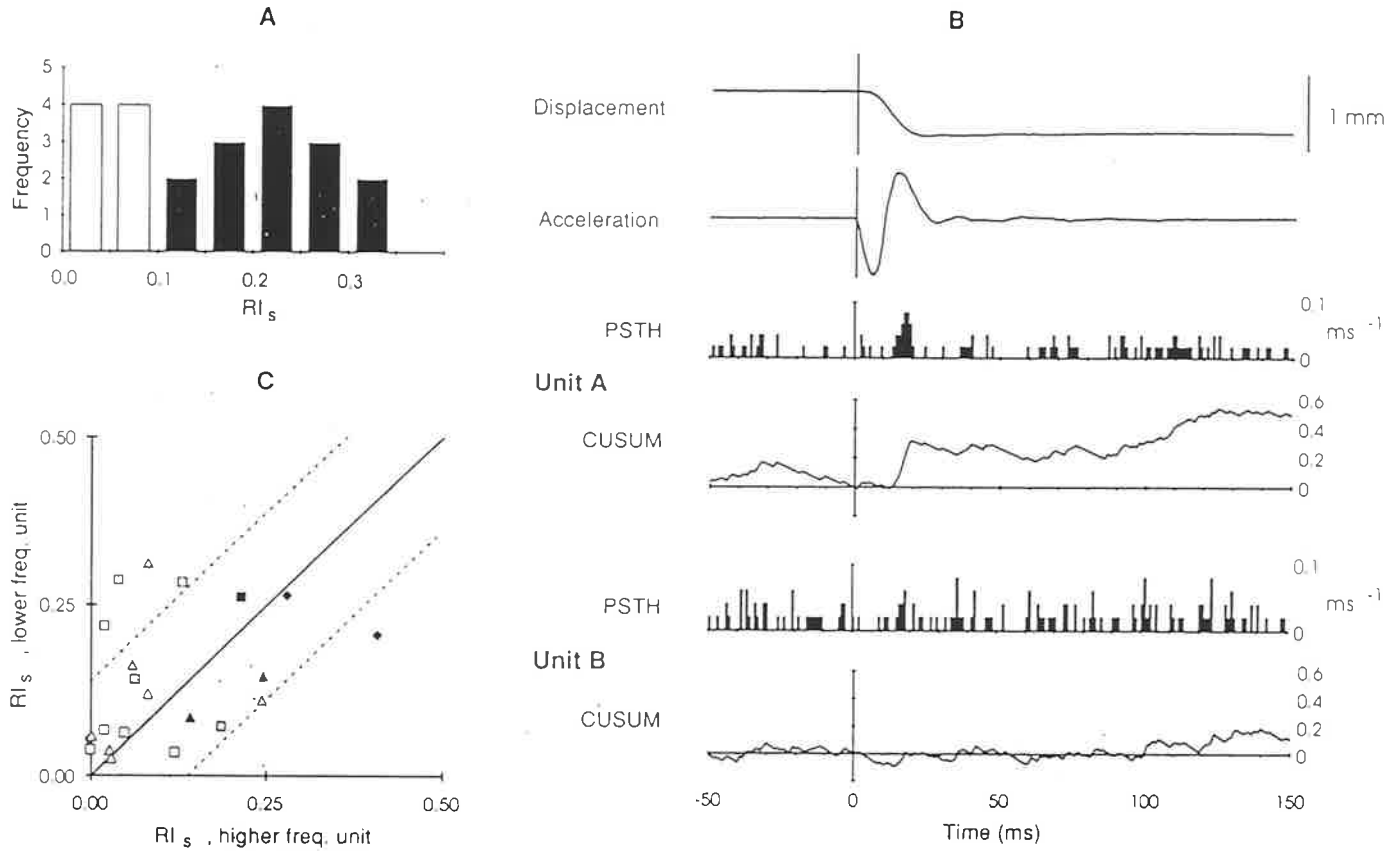

Figure 6.4 Non-uniformity of short-latency reflex responses in different masseter motor units. **A**, Distribution of RI_S values for 22 motor units tested with a stretch velocity of 31 $mm.s^{-1}$. Unfilled columns show non-significant RI_S values, filled columns show significant values. **B**, Short-latency reflex responses in two concurrently active units to a stretch of 0.5 mm, 16 ms duration. The PSTH and CUSUM of unit A show a prominent short-latency excitatory peak which is barely evident (and not significant) in unit B. **C**, Response indices (RI_S) of the short-latency reflex responses for concurrently active motor units: summary of data from experiments in which there were two or more concurrently active motor units discriminated with high reliability during stretch. The RI_S is plotted for each unit of the pair, with the unit firing at higher frequency on the abscissa, and the unit firing at lower frequency on the ordinate. Symbols represent different stretch velocities (triangle, 16 $mm.s^{-1}$; square 31 $mm.s^{-1}$; diamond, 63 $mm.s^{-1}$). The significance of RI_S values derived from the PSTH is indicated by the symbol shading (black, RI_S significant for both units of the pair; grey, RI_S significant for one unit of the pair; open, RI_S not significant for both units of the pair). Data points are from 23 unique combinations of motor unit pair and stretch velocity (11 different motor unit pairs). The solid line is the line of identity. Dashed lines shows the 95% confidence limits, and the data points lying outside the enclosed area are those of unit pairs that had significantly different ($P>0.05$) responses to the identical stretch.

The short-latency responses of pairs of concurrently active motor units to identical stretches were not uniform. The relationships between the short-latency reflex responses of 11 pairs of units that were recorded simultaneously are shown in Fig. 6.4C. The 23 data points represent unique combinations of motor unit pair and stretch velocity (11 different pairs from 7 subjects). Different stretch velocities (16 $mm.s^{-1}$, 31 $mm.s^{-1}$, 63 $mm.s^{-1}$) are indicated by different symbols (triangles, squares, diamonds). The RI_S is plotted for each unit of the pair, with the RI_S from the unit firing at the higher frequency on the abscissa, and the RI_S from the unit firing at the lower frequency on the ordinate. A tendency for similar short-latency reflex responses in both units of a pair following a stretch would result in a clustering of points around the line of identity (solid line). In Fig. 6.4C, five data points lie further than 95% confidence limits from the line of identity (four unique unit pairs in three different subjects). That is, four of 11 unique pairs of concurrently active units had significant ($P<0.05$) differences in the short-latency response index following an identical stretch.

There was no clear relationship between stretch velocity and the size of the short-latency response in different motor units. For example, some motor units shown in Fig. 6.4C responded vigorously to a slower stretch (triangles, 16 $mm.s^{-1}$), whereas others did not respond even to higher stretch velocities (squares, 32 $mm.s^{-1}$ and diamonds, 64 $mm.s^{-1}$).

The relative discharge rate of two concurrently active motor units is a reasonable indicator of their relative recruitment order under the conditions of the study (masseter is a rate-coded muscle, and recordings were typically made at force levels about 5-10% MVC), since the higher frequency unit of a pair usually had the lower recruitment threshold. In the presentation of the data in Fig. 6.4C, the response index from the unit in the pair with the higher discharge rate is plotted on the abscissa, which in most cases was the unit with the lower recruitment threshold. There was no tendency for units with higher discharge rates (lower recruitment thresholds) to have larger short-latency reflex responses, as would be expected if the distribution of Ia afferent EPSP amplitudes in human masseter motoneurons followed the size relationships established for spinal

motoneurons in animal preparations (Harrison & Taylor, 1981). Such a relationship would have been evident in Fig. 6.4C as a tendency for data points to fall below the line of identity. It should be emphasised that the differences in recruitment threshold for different units in a pair was not large. Nevertheless, there was a clear difference in responses of individual masseter units to the same stretch that was not related to motor unit size.

Analysis of the long-latency responses in the motor units showed that all of the CUSUMs increased as a result of the stretches, and particularly with slower stretches. In many cases, this increase was not significant, but the statistical analysis is conservative for the time interval of 90 ms, since it exceeds the typical ISI value (see Davey *et al.*, 1986).

6.3.5 Effect of Firing Frequency on the Reflex Pattern

The discrepancy in reflex responses in different motor units raises the issue of the effect of discharge rate on the reflex responses. Fig. 6.5 shows the reflex responses of a motor unit to the same stretch in runs in which its mean pre-stimulus discharge rate was low (13 Hz) and high (17 Hz). The PSTH and CUSUM records show that the general pattern of the reflex response remained the same at the two discharge frequencies. There was a short-latency excitation beginning 15 ms after the stimulus, followed by a silent period and a long-latency excitation starting at about 40 ms. The amplitude of the CUSUM peaks show that the increased probability of discharge above baseline in both the short and long-latency phase of the reflex was similar in both the low (RI_S, 0.09; RI_t, 0.21) and the high frequency (RI_S, 0.11; RI_t, 0.25) runs.

The ISI plots show little shortening during the short-latency phase, and greater and more-prolonged absolute shortening during the long-latency phase in the low-frequency run. When the change in ISI is normalised to the mean ISI (lowermost traces), it can be seen that the relative change in ISI was similar in both phases of the reflex in both discharge rate trials. The same pattern was seen in the other four motor units that were tested with different pre-stimulus discharge rates and the same stretch.

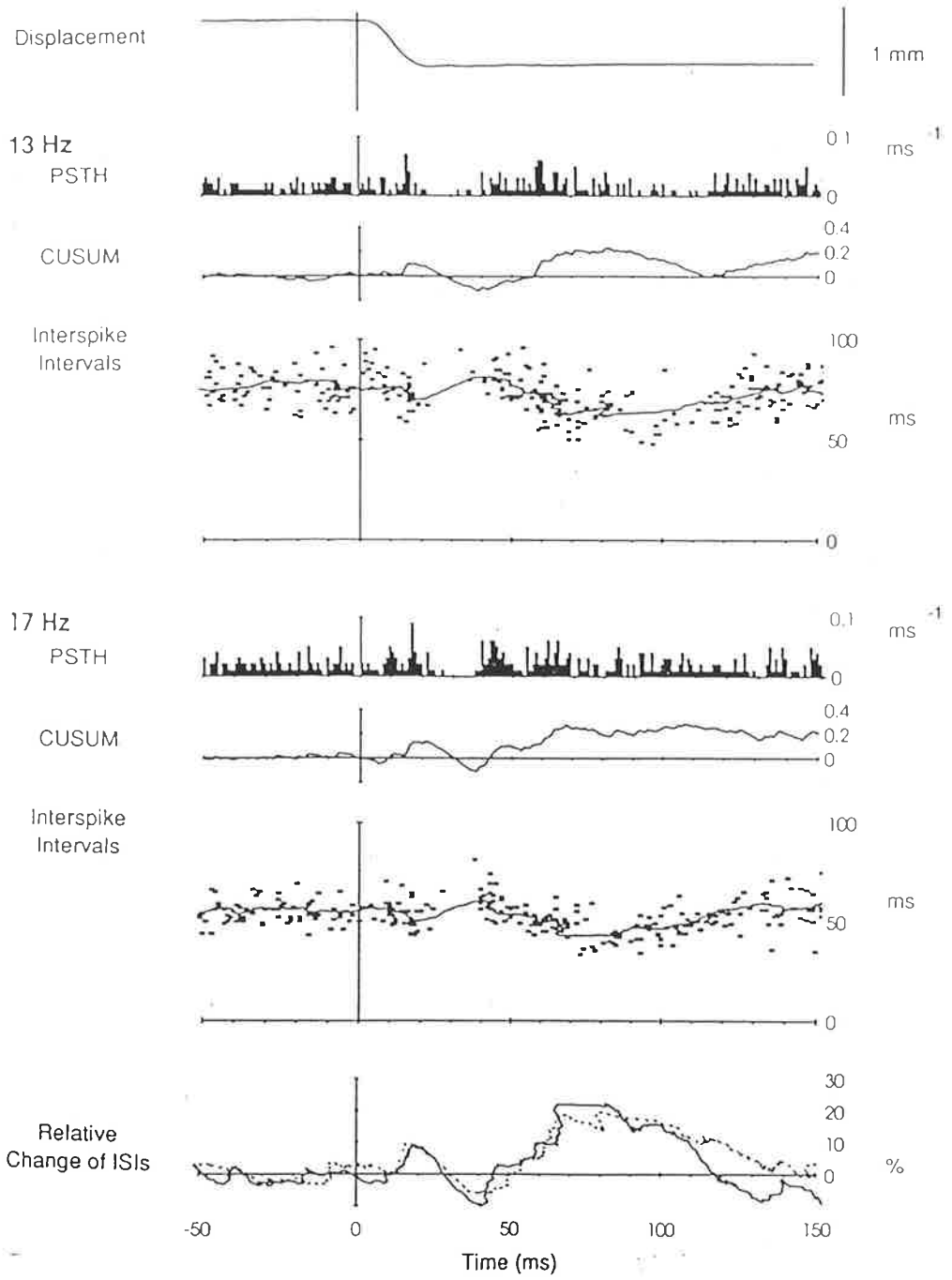

Figure 6.5 Effect of motor unit discharge rate on reflex responses. Data are from a single masseter motor unit responding to the same stretch parameters in runs in which the unit was discharging at a mean rate of 13 Hz and 17 Hz. Upper trace is jaw displacement (opening downwards). PSTH, CUSUM and ISI plots show the reflex response when the unit's mean discharge rate was 13 Hz (mean ISI=77 ms) and 17 Hz (mean ISI=55 ms). There is a similar increase in discharge probability in the CUSUM at both short and long reflex latencies at the two discharge rates. The ISI plots show little shortening during the short-latency phase, and greater absolute shortening during the long-latency phase when the unit was discharging at 13 Hz. In the lowermost traces, the changes in ISIs have been normalised as a percentage of mean ISI. The relative change in ISI was similar in both phases of the reflex at both discharge rates.

6.3.6 Stretch-induced Recruitment

The recruitment of a previously inactive masseter motor unit by the stimulus while the subject was controlling another motor unit at a target frequency was observed on only two occasions. One of these is shown in Fig. 6.6. The stretch-recruited unit was identified unambiguously because its action potentials were substantially larger than those of the other active unit. This unit was silent for the first 15 trials, then in trials 16-28 it responded intermittently in the long-latency phase of the reflex. It never discharged in the short-latency phase in trials in which it was not tonically active prior to the stimulus. From trial 29 it began discharging tonically, and was then able to discharge within the short-latency component of the reflex. The same pattern was seen in the only other case of stretch-induced recruitment that was observed.

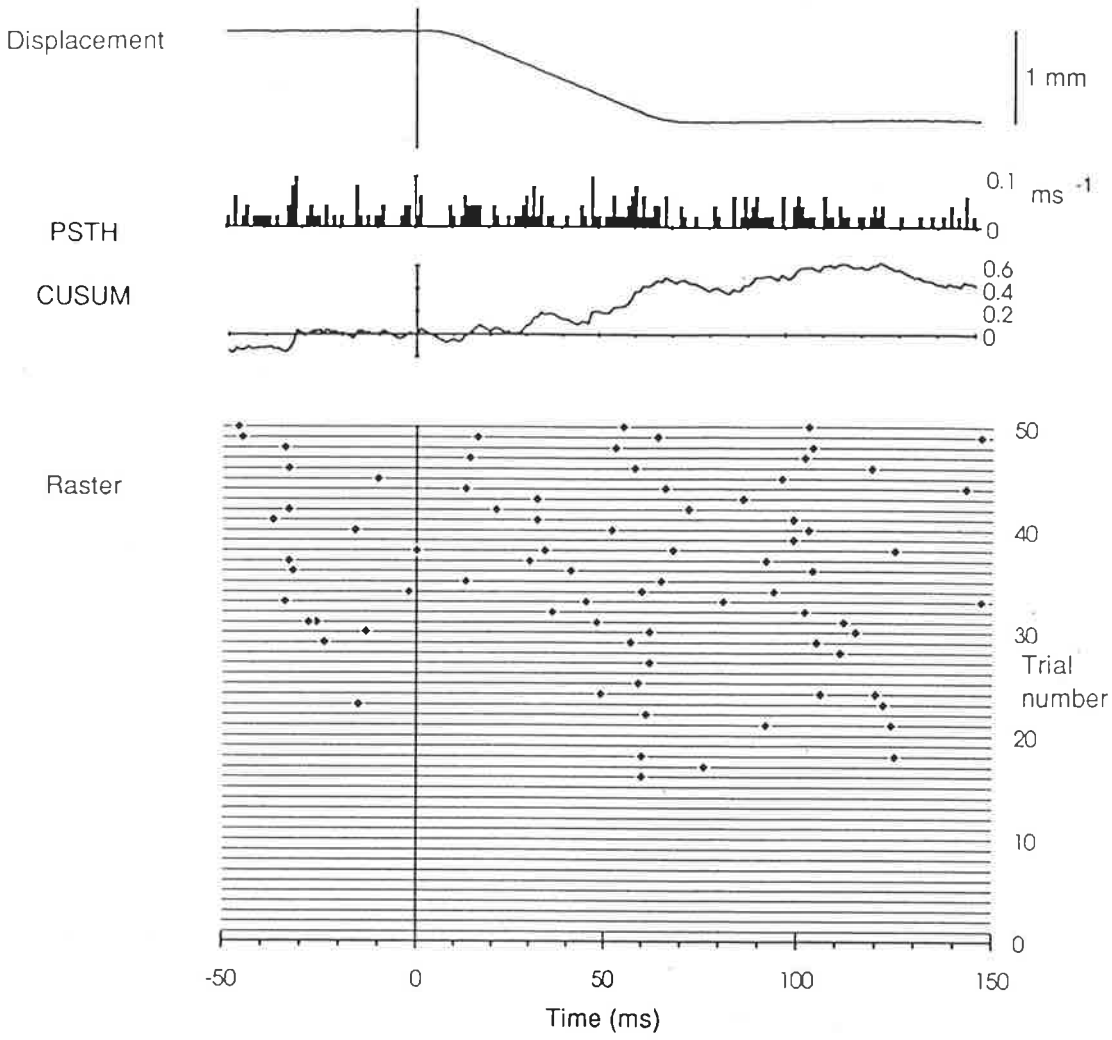

Figure 6.6 Stretch-induced recruitment of a masseter motor unit by a 1 mm stretch at 64 ms duration. A different unit was being controlled by the subject at a constant mean rate during these trials. The raster shows that this unit was not tonically active until about trial 29. The first response to the stretch stimulus occurred in trial 16, and from this trial until it became tonically active, all reflex responses occurred in the long-latency phase. Once the unit became tonically active, some discharges were seen at short latencies following the stimulus.

6.4 DISCUSSION

6.4.1 General Features of the Motor Unit Responses to Stretch in Human Masseter

Until recently, it was believed that the jaw muscles responded to stretch with only a short-latency excitation, which was followed by a silent period. The apparent absence of the long-latency stretch reflex response led to the suggestion that the jaw muscles lacked the long-latency, presumably transcortical, reflex loop that is prominent in limb muscles (Lamarre & Lund, 1975; Goodwin, Hoffman & Luschei, 1978; Cooker, Larson & Luschei, 1980). It has been shown in Chapter 4 that smooth stretches of the human masseter muscle produce reflex excitation of the muscle that consists of two phases of excitation beginning at latencies of around 10 ms (short) and 35 ms (long) in the surface EMG (see Chapter 4 and Poliakov & Miles, 1994). These are separated by a period of reduced activity starting at about 20 ms, which represents the silent period, but is also comparable in latency with the inhibitory pathway from the periodontal receptors (Linden, 1990; Brodin, Türker & Miles, 1993).

There is limited information regarding single motor unit reflex responses in a human muscle undergoing prolonged stretch (suitable for producing long-latency reflex responses), probably because of the technical difficulties involved in recording in these conditions. Bawa & Tatton (1979) initially proposed that separate populations of motor units responded in the short- and long-latency phases of the reflex. This conclusion was not supported in a later study in which they reported that tonically active motor units could respond in either short or long-latency phases of the reflex, and that non-tonically active units responded preferentially in the long-latency phase (Calancie & Bawa, 1985a). The same authors reported that the recruitment order was the same for motor units activated by voluntary effort and the stretch reflex (Calancie & Bawa, 1985b). Palmer & Ashby (1992) have presented evidence that the long-latency stretch reflex in human *flexor*

pollicis longus involves a transcortical pathway by testing convergence onto cortical neurons with magnetic brain stimulation.

The present report shows that the general features of motor unit reflex responses to stretch are similar in the masseter to those in other muscles, with short- and long-latency excitation separated by a silent period. Obvious differences, however, are the much shorter latencies of both phases of the reflex in the masseter and the highly synchronous discharge in the short-latency phase for masseter units. These two features can be explained by the short afferent and efferent distances for the reflex arcs in the jaw muscles. The latency of the H-reflex in the masseter is about 5.5 ms (Godaux & Desmedt, 1975a), compared with 7.5 ms for the short-latency stretch response. The highly synchronous discharge in the short-latency phase is assisted by the short distances involved in the reflex arc that minimise temporal dispersion of the responses of different motor units due to differences in conduction velocity. However, there is some question about the pathway of the long-latency response in masseter. Non-painful trigeminal stimuli evoke a potential over the sensory cortex that peaks at about 20 ms (Findler & Feinsod, 1982), and the efferent conduction time from the motor cortex to the masseter is about 6 ms (Cruccu, Berardelli, Inghilleri & Manfredi, 1989). Thus its latency of onset (35 ms) is still some 9 ms longer than the minimum calculated transcortical loop time.

The present findings in masseter support the conclusion of Calancie & Bawa (1985a) that tonically active motor units can respond in both the short and long-latency phase of the reflex. The reason for this is now clear from the sorted raster plots (e.g., Fig. 6.1). The unit will not discharge in the short-latency phase of the reflex in trials in which it has discharged just prior to the stimulus. This is because the motoneuron membrane potential is too far from its firing threshold in the early part of the ISI for the short-latency compound EPSP to evoke an action potential. It is only when the stimulus is timed so that the compound EPSP (either the short- or long-latency component) occurs when the membrane potential is close to threshold that an action potential will result.

The present study also confirms the observation of Calancie & Bawa (1985a) that non-tonically active units respond preferentially in the long-latency phase of the reflex. The reason for this must be the shape of the compound EPSP evoked by the stimulus, with the short-latency component being smaller than the long-latency phase. This suggestion is supported by the ISI *vs* time plots (Figs. 6.1, 6.3, 6.5) which show that the shortening of the ISIs is greater and more prolonged during the long-latency phase of the reflex. Indeed, this representation of the data provides an estimate of the shape and time-course of the profile of the underlying compound EPSP (Chapter 5).

6.4.2 Stretch Velocity and Single Motor Unit Responses

The differing responses of individual motor units to slow and fast stretches are consistent with the pattern seen in the surface EMG (Chapter 4 and Poliakov & Miles, 1994). With slow stretches, units responded predominantly with a long-latency excitation, which changes to predominantly short-latency excitation at the faster stretch velocities (Fig. 6.3). The overall response (RI_t) is greater with slow stretches (Fig. 6.3C). Calancie & Bawa (1985a) found a similar pattern for the wrist extensors with increasing torque step load. There was a shift from long- to short-latency for non-tonic units. For tonic units, the response probability during the short-latency period increased monotonically with load; long latency responses decreased at higher loads. In contrast with our findings, they found an increase in the unit's total response probability during the overall reflex period with higher torque loads.

The ISIs *vs* time analyses of motor unit discharge (Fig. 6.3, lower trace) explains our earlier observation that the reflexly evoked force declines as the rate of stretch in the masseter increases (Chapter 4 and Poliakov & Miles, 1994). In that previous report, we debated why the long-latency response produced more force than the short-latency response, despite the prominent short-latency peak in the surface EMG. Amongst other things, we speculated that this may be due to the asynchronous recruitment of additional motoneurons by the cortico-trigeminal pathway during the long-latency phase. The

responses of single motor units observed in the present study reveal that this is not a major factor, as stretch-evoked recruitment was rarely seen in the intramuscular records. The principal difference in responses evoked by slow and brisk stretches was the greater modulation of motor unit ISIs over a longer time period with the slow stretches. For example, in Fig. 6.3B the brisk stretch of 8 ms duration produced minimal shortening of ISIs in the short-latency phase, and a maximum shortening of about 20% in the long-latency phase. With the slower stretch (Fig. 6.3A), the shortening of ISIs was much larger (45%) and more prolonged. The synchronous peak in the PSTH is misleading in relation to the size of the force response, because there is little shortening of ISIs in the short-latency period. It is the *change* in ISIs that determines the reflexly-evoked force.

Pooling the data from all subjects revealed a positive correlation between the size of the short-latency reflex response in motor units and the size of their long-latency reflex responses. On the available data, it is not possible to say to what extent this relationship is influenced by subject differences in responsiveness to stretch. This question can be answered by sampling a large number of masseter motor units in a single experiment, and testing with two standard stretch velocities.

6.4.3 Discharge Rate and Single Motor Unit Responses to Stretch

The pre-stimulus discharge rate of the motor unit did not alter the response profile evoked by stretch. The normalised PSTH and CUSUM were similar at different motor unit discharge rates with the same stretch (Fig. 6.5). This confirms earlier observations for H-reflex responses in human soleus motor units by Ashby & Zilm (1982) and Miles, Türker & Le (1989), and also is in general agreement with the concept of "automatic gain control" (Marsden *et al.*,1976). The ISIs *vs* time plots show that the *relative* change in ISI evoked by a stretch was constant at different mean discharge rates (Fig. 6.5, lower traces). This is consistent with our recent suggestion that the normalised change in ISIs with time reflects the shape of the post-synaptic potentials arising in the motoneuron as a result of the stretch (Chapter 5). That is, one would not expect the shape of the EPSP

evoked by a given stimulus to change when the unit was discharging at different pre-stimulus firing rates.

In Fig. 6.5, the steady biting force was 20 N in the low-frequency trial and 30 N in the high-frequency trial. This corresponds to an increase from approximately 10% to 15% of maximal incisal biting force. The similarities of the reflex responses in the two trials in the same motor unit also suggest that spindle responsiveness to the stretch was not markedly altered by changes in fusimotor drive over this force range in this subject.

6.4.4 Muscle Spindle Projections to Motoneurons in Human Masseter

In the cat hindlimb, individual spindles project very widely within motor pools to evoke EPSPs in nearly all (85-100%) homonymous motor neurons (Mendell & Henneman, 1971; Watt *et al.*, 1976). The EPSPs from primary spindle afferents are larger in small motoneurons (Harrison & Taylor, 1981). In humans, the inputs of spindle Ia afferents to individual motoneurons have been assessed indirectly from PSTHs of motor unit discharge following both H-reflex stimuli and tendon taps. In the study by Bayoumi & Ashby (1989), 29/30 (97%) of motor units tested in various thigh muscles showed facilitation at H-reflex latency following stimulation of the homonymous muscle nerve. All 93 motoneurons sampled by Mao, Ashby, Wang, & McCrea (1984) in human tibialis anterior, medial gastrocnemius and soleus were facilitated at H-reflex latency by electrical stimulation of their muscle nerves. All 32 motor units studied by Buller, Garnett & Stephens (1980) in the human first dorsal interosseus were facilitated at monosynaptic latency by a brief mechanical pulse applied to the belly of the muscle. The conclusion from the human and animal studies is that in all spinal motor pools tested, virtually all motoneurons receive short-latency excitatory inputs from homonymous muscle spindle afferents.

In the jaw muscles, however, the situation is clearly different. Only 10% of cat masseter motoneurons receive an input from a single Ia afferent (Appenteng *et al.*, 1978),

suggesting that the projection frequency is much weaker for Ia afferents in the trigeminal system than in spinal muscles, where it approaches 100% (Mendell & Henneman, 1971; Watt *et al.*, 1976). When present, unitary EPSPs in jaw-elevator motoneurons from single spindle afferents were small (mean amplitude 18.3 ± 51.4 μV), in comparison with those in spinal motoneurons (Appenteng *et al.*, 1978). Electrical stimulation of the masseteric nerve at an intensity just below the motor threshold elicited small compound EPSPs in all masseter motoneurons in the six cats tested, although these were small (mean 0.72 mV). These data suggest that the majority of masseter motoneurons in the cat receive some monosynaptic excitatory input from muscle spindles but that the effect is weak, in part because of the low projection frequency of individual spindle afferents within the motor pool. Limited homonymous projection frequency (30%) and small unitary EPSPs (mean 21.5 μV) were also found for spindle afferent inputs to the trigeminally-innervated lateral pterygoid motoneurons in the guinea pig (Nozaki *et al.*, 1985).

In the present study, 8 of 22 (35%) masseter units showed no significant short-latency response to even the fastest stretch velocities (32 $mm.s^{-1}$, 64 $mm.s^{-1}$) which evoked prominent short-latency reflex responses in the masseter surface EMG in all subjects. The velocity threshold for short-latency reflex responses was below 16 $mm.s^{-1}$ in 13 of 22 units (60%). The responses of concurrently active motor units to the identical stretch within a single experimental run were often quite different (Fig. 6.4). It is likely that a stretch of 1 mm at these velocities modulates the discharge of all primary spindle afferents in the masseter (Larson, Smith & Luschei, 1981). Detection of connectivity is enhanced in situations where the motoneuron is tonically active, as compound EPSPs that are not capable of reaching threshold in the resting motoneuron are able to modulate the discharge of the tonically active neuron when the membrane potential is on average closer to its firing threshold. Although the PSTH technique may not detect small EPSPs (Kirkwood, 1979; Cope, Fetz & Matsumura, 1987), it is safe to say that 35% of the masseter motoneurons examined received no functional short-latency excitatory input

from jaw muscle spindles. In that regard, the masseter is quite different to all muscles in which this question has been examined to date.

The finding that 35% of masseter units lacked a significant short-latency response to stretch raises the question whether different types of masseter motor units are less likely to receive monosynaptic inputs from muscle spindles. The recruitment order did not appear to be an important factor. For concurrently active motor units, the relative discharge rates are related to recruitment force threshold (Tanji & Kato, 1973; Monster & Chan, 1977), and in the masseter there was no clear relationship between the relative discharge rate of motor units and the size of the short-latency reflex response (Fig. 6.4). It should be noted, however, that most of the units in this study were low-threshold motor units (recruitment thresholds below 15% of MVC), and the differences in recruitment threshold of two units in a pair were not large.

The masseter is a complex multipennate muscle with regional differences in fibre angulation (Baron & Debussy, 1979) and histochemical fibre types (Eriksson & Thornell, 1983). The finding that motor units of similar recruitment threshold can have large differences in their short-latency reflex responses to stretch raises the issue of reflex partitioning (*c.f.* Windhorst, Hamm & Stuart, 1989); that is, whether the relative proximity of the motor units within the muscle influences the similarity of their reflex responses. On the basis of surface EMG recordings, Lobbezoo, van der Glas, Buchner, van der Bilt & Bosman (1993) have suggested that the monosynaptic reflex response to jaw-jerks is greater in the deep compared with superficial parts of the masseter. Our data suggest that the differences in short-latency reflex responses in different motor units were not specifically influenced by the location of the motor units within the muscle. The short-latency responses of motor unit pairs recorded either from the same electrode (e.g. Fig. 6.4B) or from separate electrodes were sometimes significantly different. It may be worthwhile, however, to evaluate this issue systematically in the masseter using electrode locations that are more widely spaced.

In summary, the pattern of reflex response in a single motor unit depends on the rate and amplitude of stretch, and the timing of the stimulus with respect to the preceding discharge in a tonically active motor unit. The motor unit discharge rate does not affect the reflex response. Individual motor units in the human masseter do not respond uniformly to smooth jaw displacements. While tonically active motor units were found that exhibited phases of excitation consistent with the overall reflex pattern in the surface EMG, a substantial proportion (35%) had weak or non-detectable short-latency excitation. There was a ten-fold range in the size of the short-latency response to the same stretch parameters in different units. Pairs of concurrently active units could have significant differences in their short-latency responses to stretch within the same experimental run. Thus, it appears that the Ia afferent inputs are not distributed uniformly among human masseter motor units. Furthermore, in pairs of concurrently active low-threshold masseter motor units, the relative recruitment order is not a good predictor of the size of the short-latency reflex.

CHAPTER 7

CONCLUDING REMARKS

7.1 An Overview, with the Physiological Implications of this Study

This thesis presents a detailed study of the organisation and role of the stretch reflex in the jaw-closing muscles of healthy humans. The reflex responses to slower stretches were a particular focus of this study, since there have until now been no such experimental data for masticatory muscles. In contrast, reflex responses to slow stretches have been studied intensively in many limb muscles over the last thirty years, revealing the important role of the long-latency phase of the stretch reflex. The main aim of this study was to investigate the organisation of the stretch reflex in jaw-closing muscles and to compare it to that in the other skeletal muscles. To achieve these goals, I employed a number of approaches, both experimental and theoretical.

The special-purpose high-performance stretcher was designed and built in the first stage of this project. It incorporated a high-performance mechanical system driven by a powerful motor, which was controlled by a specially designed control system. The stretcher delivered precise jaw displacements, specified by a special-purpose computer program, without vibrations or ripples in the displacement trajectory. The design, characteristics and performance of the stretcher are described in detail in Chapter 2, along with the preliminary physiological data obtained.

Using theoretical considerations and mathematical analysis, I developed a novel approach to treatment of the surface EMG signal for reflex studies, namely the method of the

integral of the averaged EMG. Its mathematical justification, experimental verification, scope of application, advantages and limitations are presented in Chapter 3. This method was used at all stages of this study, and result obtained with this technique are presented in Chapters 4, 5 and 6.

It is well known that the reflex response to stretch in most contracting human muscles includes both a short-latency, probably monosynaptic, excitatory component, as well as a longer-latency, polysynaptic excitation. However, it had been claimed that stretch of the jaw-closing muscles evokes only the short-latency response in masseter. If true, this would have implications for the role of the stretch reflex in motor control, as the jaw muscles perform highly coordinated movements. I re-examined this question in a series of experiments, using controlled stretches of varied rates and durations.

In the first stage of the experimental study I employed the surface EMG technique, which provides information on the activity of the whole muscle. Other physiological parameters recorded were biting force, displacement and acceleration pattern. These experiments are described in Chapter 4, and the major findings can be summarised as follows:

1. Very brief, rapid stretches analogous to the stimuli used to investigate the "jaw-jerk" reflex in earlier studies evoked a prominent excitatory peak in the electromyogram at monosynaptic latency excitation, but little or no longer-latency excitation. This response could be produced even by stimuli that were barely detectable by the subject. However, this prominent electrical response did not produce a measurable increase in biting force.

2. In contrast, slower stretches evoked both a short- and a longer-latency excitatory response in the surface electromyogram, as in most limb muscles. It was shown that the absence of a long-latency excitatory response in earlier studies can be explained by the powerful reflex disfacilitation of the motoneurons that occurred at the end of the brief stretches used. Depending on the duration of the stretch, this disfacilitation is often sufficient to mask or abolish the long-latency reflex.

3. The reflex response to stretches was not markedly affected by blocking the activation of mechanoreceptors around the teeth with local anaesthetic, indicating that receptors around the teeth cannot be playing more than a minor role in the response.

4. The stretch-induced increase in force became greater as the velocity of the stretch decreased.

After studying the reflex at the level of the whole muscle, using the surface EMG technique, I expanded the experiments to the level of individual motoneurons, using intramuscular EMG recording. A methodological question, arising from the single motor unit technique, is discussed in Chapter 5. In this chapter I have analysed mathematically the simple threshold-crossing motoneuron model and suggested a new method for *reconstruction of the compound post-synaptic potential* (PSP), evoked by a stimulus, from the plot of the interspike intervals (ISIs). I present examples of the application of this method to experimental data in Chapters 5 and 6. In the Methods section of Chapter 6 I have introduced a novel method for displaying spike-train data obtained in a reflex paradigm, namely, the *sorted raster*. This method is applicable to study the pattern of response of single neuron to a stimulus delivered randomly, and essentially is a way of presenting the data as a raster, with individual trials re-arranged. With this analysis, the dependence of the neuron's response on the timing of the last pre-stimulus discharge becomes clear. Its application to the response of masseter motoneurons to stretch is presented in the Results section of Chapter 6.

The second stage of the experimental study was focused on the reflex pattern of individual motoneurons. The single motor unit technique was employed along with the parameters recorded in the first stage of this study. Reflex responses of individual motoneurons were of particular interest, since there are no data available for masseter motoneurons. In fact, there is a very limited number of studies and amount of experimental data on the behaviour of individual motoneurons of any muscle in response

to stretch because of technical problems of stability of the intramuscular EMG recording in the muscle undergoing stretch. Therefore, the results obtained in these experiments and described in Chapter 6, are also of more general interest for motor control studies and stretch reflex organisation. The major results of these experiments are summarised here:

1. The majority (65%) of tonically active masseter motor units were excited in both short- and long-latency phases of the reflex. The timing of the stimulus in relation to the preceding discharge of the motor unit determined whether the unit discharged in the short- or long-latency phase of the reflex. If a non-tonically active motor unit was recruited by the stimulus, it invariably discharged in the long-latency phase.

2. Short-latency responses were strongly time-locked to the stimulus in both the surface EMG and the motor unit peristimulus time histogram (PSTH). Despite the prominence of these short-latency peaks, there was very little shortening of interspike intervals (ISIs) in the short-latency phase of the reflex. The shortening of ISIs was more prominent and prolonged during the long-latency phase of the reflex, which explains why the long-latency reflex contributes most of the reflex force changes following the stretch.

3. Pairs of concurrently active motor units could have significant differences in their short-latency responses within the same experimental run. There was a ten-fold range in the size of the short-latency response to the same stretch in different motor units.

4. Although a prominent short-latency response was evident in the surface EMG of all 7 subjects tested, a substantial proportion (35%) of the 22 masseter motor units tested had no statistically significant short-latency reflex response.

5. It is concluded that the short-latency, presumably monosynaptic, Ia afferent inputs to human masseter motoneurons are not uniformly distributed amongst all

motoneurons in the pool. For some motoneurons, a functional connection between Ia afferents and motoneurons was not demonstrated.

To implement the novel methods, along with the conventional techniques, I developed several software packages using Microsoft Excel® 4 (see Appendix A). These programs and their modifications also allowed me not only to implement conventional and novel methods for display and analysis of my data, but also to produce automatically high quality hard copies of the experimental results in a convenient form, to calculate various parameters and apply statistical tests to the experimental data. In this laboratory, these programs now form part of the standard analyses used in other research projects.

In summary, this study presents a detailed investigation of stretch reflex mechanisms in human masseter muscle at the levels ranging from individual motoneurons to the overall muscle response. Contrary to the view prevailing until now, it was shown that the stretch reflex in the jaw-closing muscles generally consists of the short-latency phase, followed by a long-latency phase, which is similar to the pattern found in many limb muscles. The physiological importance of the long-latency phase has emerged as a result on numerous studies in spinal systems. In agreement with this, by using slower stretches in individual masseter motoneurons, I found that the long-latency phase of the reflex is the force-producing phase. I have demonstrated that the force is the result of a substantial decrease of ISI values over a prolonged period of time during the long-latency phase of the reflex. On the other hand, the short-latency phase may be the manifestation of synchronisation of motoneuron discharges, which is not accompanied by any substantial decrease of ISIs. Moreover, in one third of masseter motoneurons studied, the short-latency phase was insignificant. The implication of these results is that the long-latency phase of the stretch reflex should be regarded as playing the dominant role in the reflex control of the jaw-closing muscles, and is broadly similar to that in limb muscles.

This study also contributes to the methodology of motor control studies. Three new quantitative methods of analysis of reflex events in motor systems have been described that have general value for studies of motor function at the levels of individual motoneurons and the whole muscle.

7.2 Unanswered Questions and Future Developments

Let us consider what questions remained unanswered and what further developments may be suggested as a result of this study. In the limb muscles, the stretch reflex has been studied using a variety of approaches, including the dynamic response to periodical or random changes of muscle length. This question is yet to be studied in the masticatory system. An important property of the long-latency phase of the stretch reflex in limb muscles is that it can undergo substantial modifications depending on the "functional set" or modification of the motor task (Houk & Rymer, 1981), and provides a coordinated muscle response to stretch (Gielen, Ramackers & van Auylen, 1988). It is likely that the cortex and the transcortical pathways are involved in these phenomena. Because it is not yet clear whether the pathway of the long-latency reflex in the jaw-closing muscles traverses the cortex, it would be interesting to determine whether the long-latency phase is modified by "functional set" in the same way. Superimposing the stretches onto much slower ramps or sinusoidal displacements, more closely analogous to chewing cycles, may also reveal the role of the stretch reflex and its long-latency phase for coordinated muscle response in a more physiological context (Ottenhoff, van der Bilt, van der Glas & Bosman, 1992). This might be of particular interest in the masticatory system since it is known to have two distinctly different modes of operation, *viz* mastication and maintaining posture, and in humans, the speech function should also be considered.

The other question, that was not considered in detail in this study is the reflex response to unloading in the jaw-closing muscles. One experimental record is presented in Chapter 4 and shows the reduction of muscle activity occurring in two phases at latencies corresponding to that of short- and long-latency of the stretch reflex, but this should be studied quantitatively and in much more detail. One of the unanswered questions is whether the stretch and unloading reflexes are symmetrical within a certain narrow range of amplitudes and rates of the displacement pattern.

When the receptors contributing to the origin of the stretch reflex are considered, it seems clear that muscle spindles play the major role, although our understanding of properties of these and other receptors could be more detailed. The neural pathways involved in the long-latency phase of the reflex remains mainly speculative. By analogy with the spinal systems, one would expect that the motor cortex would play an important role (see Matthews, 1991). However, the latency of this phase in the jaw-closing muscles exceeds that of the shortest possible transcortical loop. Some insight into the role of motor cortex in formation of the long-latency phase may be gained by conducting experiments analogous to those of Palmer & Ashby (1992), who used transcranial electromagnetic stimulation of the cortex to demonstrate the involvement of the motor cortex in the long-latency phase in spinal motor units.

Several novel methods of analysis of experimental data obtained in the reflex paradigm are described in this thesis. As it has been shown here, there is a non-trivial difference between these and the conventional methods of analysis. For example, the method of the integral of the averaged EMG gives a *quantitative* estimate of the reflex, which the conventional method of averaged rectified EMG does not (see Chapter 3). The method of reconstruction of the compound PSP from the ISIs values reveals and describes *quantitatively* the phases of excitation and/or inhibition in a motoneuron and, unlike the conventional methods of presenting the data as PSTHs and their CUSUMs, avoids masking of these phases by secondary peaks or troughs (see Chapter 5). The application of these new methods to studies of the human nervous system should reveal important, quantitative insights to reflex function in health and disease.

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APPENDICES

APPENDIX A

Software Developed for Processing of the Experimental Data

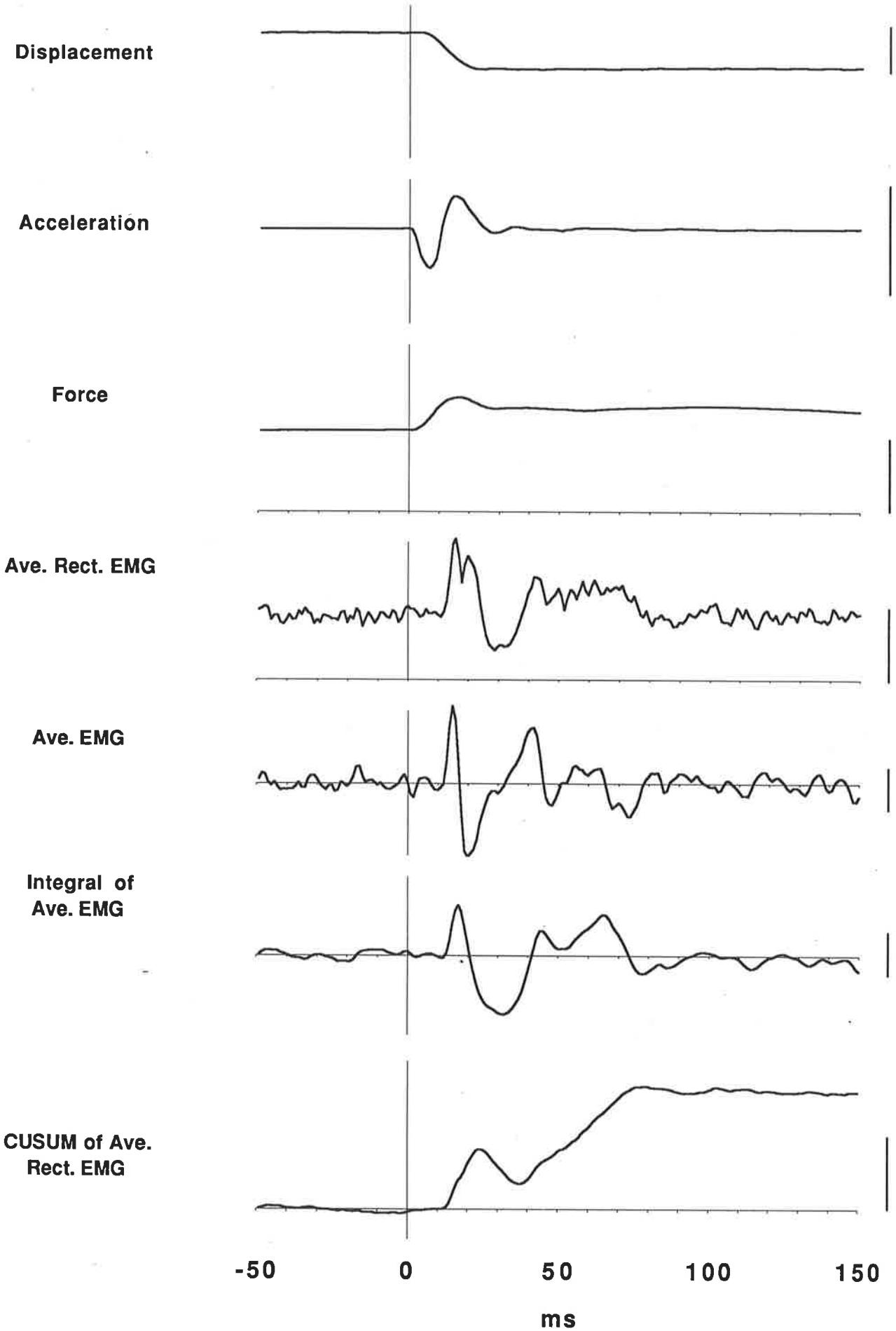
The printouts of the spreadsheets and programs for analysis and processing of the data for the experiments described in this thesis are listed herein. These were developed for **Microsoft Excel® 4** to organise data in the spreadsheets, perform simple calculations using the spreadsheet facilities, and to present the results in the graphic form as charts. The **Macro** language of Excel was used to develop programs for automatic data processing: loading data from the data files into the spreadsheets, performing more complex computations and making hard copies of high quality in the illustrated form.

Workbook **BOOK1.XLW** was developed for the experiments with surface EMG recordings, and it consists of a spreadsheet and a program. **Page 1** of the spreadsheet **RFPE.XLS** shows the charts of some experimental data averaged for all trials: displacement, acceleration, force, averaged rectified EMG, averaged EMG. The integral of averaged EMG and CUSUM of averaged rectified EMG were calculated using the spreadsheet facilities. The data for these charts are organised in the **page 2** and the following pages (not shown) of the spreadsheet. The program was used to load the data from the data files into the spreadsheet and was activated by opening a data file and pressing **Cntr+l**. Pressing **Cntr+b** activated the batch mode of the program, which loaded and printed out all files of one experiment.

Workbook **MU.XLW**, consisting of a spreadsheet and a program, was used to analyse and print out the data of the intramuscular experiments with single motor unit registration. Raster files were obtained at the preliminary phase of the analysis using the methods available in the laboratory and were used as input files for the program. These data were loaded into the spreadsheet **MU.XLS** by the program **MU.XLM**. The program was activated by pressing **Cntr+l**. It also performed calculations for the PSTHs and sorted rasters, and printed out **page 1** of the spreadsheet, which shows the charts of PSTH, its CUSUM, Plot of ISI Values, Raster and Sorted Raster.

These workbooks were the basis for many modifications, which are not presented here.

Data file:01tm0102.a



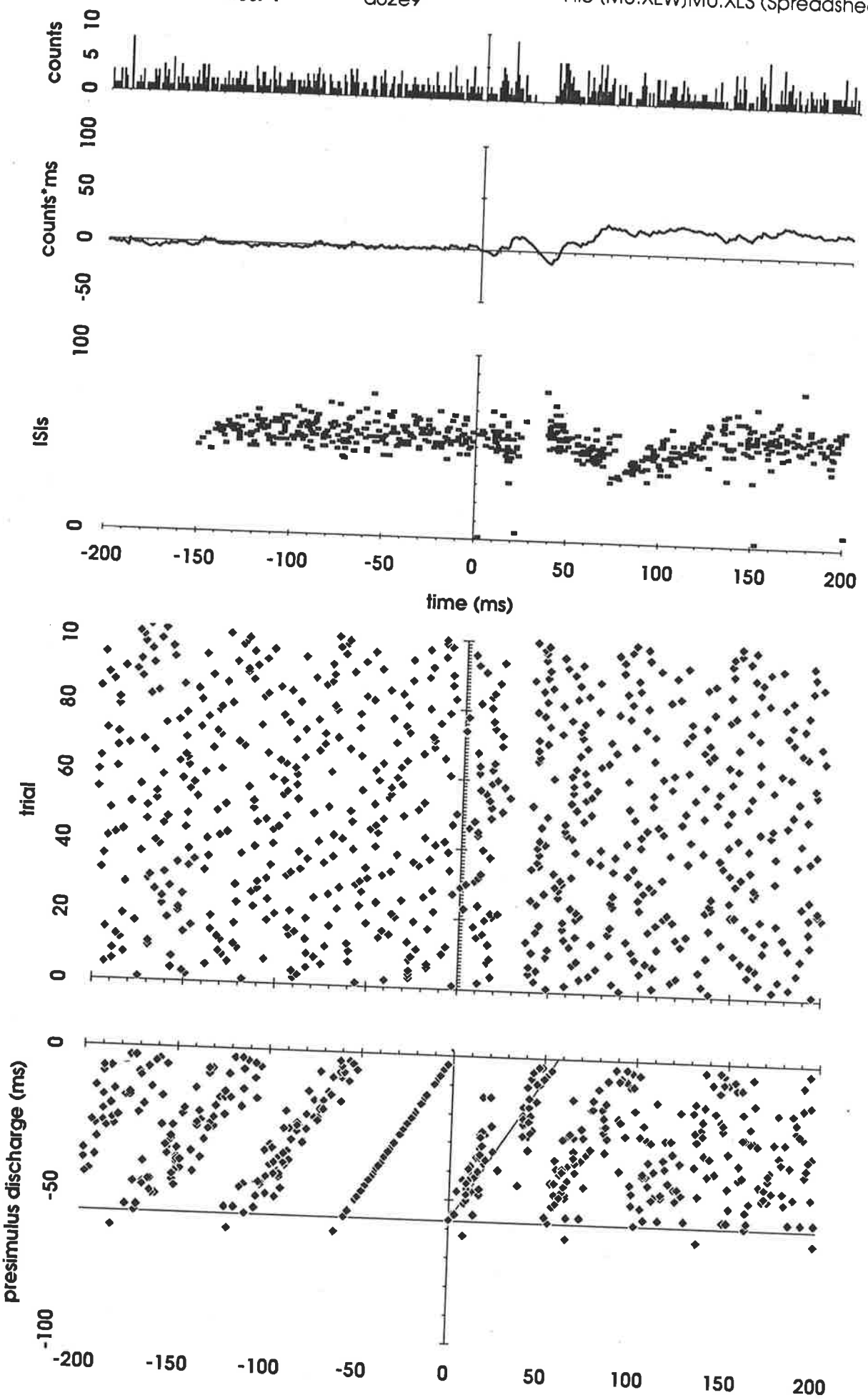
Time		REMG	acceleration	force	position	EMG	CUSUM	R_CUSUM
	-50							
	-49	0.25281	-0.145288	0.72324	0.83264	0.02235	2.35E-02	2.03E-02
	-48	0.25935	-0.14502	0.72361	0.83237	0.0556	8.02E-02	4.72E-02
	-47	0.26807	-0.14519	0.72412	0.8323	0.04559	1.27E-01	8.28E-02
	-46	0.26043	-0.144824	0.72395	0.83345	0.00721	1.35E-01	1.11E-01
	-45	0.21737	-0.145361	0.72437	0.83345	-0.0113	1.25E-01	9.57E-02
	-44	0.23346	-0.145166	0.72429	0.83318	0.00674	1.33E-01	9.67E-02
	-43	0.23256	-0.145093	0.72424	0.83337	-0.0204	1.14E-01	9.68E-02
	-42	0.20732	-0.1448	0.72478	0.83481	-0.0392	7.57E-02	7.17E-02
	-41	0.20085	-0.145044	0.72478	0.83479	-0.0319	4.50E-02	4.01E-02
	-40	0.21677	-0.145117	0.72444	0.83423	-0.037	9.11E-03	2.44E-02
	-39	0.23478	-0.145386	0.72476	0.83389	-0.0329	-2.26E-02	2.67E-02
	-38	0.21779	-0.145361	0.72478	0.8343	-0.0324	-5.39E-02	1.20E-02
	-37	0.21863	-0.145313	0.72449	0.83191	-0.0182	-7.09E-02	######
	-36	0.24829	-0.145313	0.72476	0.83198	0.0036	-6.61E-02	1.40E-02
	-35	0.23579	-0.145239	0.725	0.8332	-0.0234	-8.83E-02	1.73E-02
	-34	0.25743	-0.145117	0.72446	0.83298	-0.0244	-1.12E-01	4.23E-02
	-33	0.24108	-0.145117	0.72476	0.83381	0.01936	-9.11E-02	5.09E-02
	-32	0.23694	-0.145361	0.725	0.83325	0.0419	-4.81E-02	5.54E-02
	-31	0.202	-0.145239	0.72488	0.83403	0.04064	-6.29E-03	2.49E-02
	-30	0.21652	-0.145386	0.72537	0.83535	0.02324	1.81E-02	9.00E-03
	-29	0.2049	-0.145166	0.72529	0.8345	-0.0083	1.10E-02	######
	-28	0.2338	-0.144775	0.72598	0.83369	-0.0255	-1.34E-02	######
	-27	0.23014	-0.144824	0.72581	0.83369	-0.0372	-4.94E-02	######
	-26	0.22647	-0.145044	0.72583	0.83267	-0.0127	-6.10E-02	######
	-25	0.21837	-0.145605	0.7261	0.83235	-0.0132	-7.30E-02	######
	-24	0.22057	-0.144873	0.72583	0.83201	-0.0456	-1.17E-01	######
	-23	0.21348	-0.1448	0.72656	0.83311	-0.0379	-1.54E-01	######
	-22	0.24202	-0.144873	0.72683	0.83403	0.00626	-1.47E-01	######
	-21	0.24633	-0.144824	0.72654	0.83286	-0.0032	-1.49E-01	######
	-20	0.2059	-0.144995	0.72681	0.83442	-0.0162	-1.64E-01	######
	-19	0.22955	-0.14541	0.72664	0.83472	0.00137	-1.61E-01	######
	-18	0.21816	-0.145483	0.72695	0.83457	0.03793	-1.22E-01	######
	-17	0.25574	-0.145142	0.72759	0.83394	0.08904	-3.21E-02	######
	-16	0.24307	-0.144922	0.7273	0.83386	0.09382	6.29E-02	######
	-15	0.19194	-0.145337	0.72712	0.83364	0.03751	1.02E-01	######
	-14	0.22036	-0.145215	0.72759	0.83184	0.00715	1.10E-01	######
	-13	0.24121	-0.145239	0.72715	0.8325	0.01129	1.22E-01	######
	-12	0.22598	-0.145215	0.72759	0.83269	0.01616	1.40E-01	######
	-11	0.20381	-0.145093	0.72783	0.83303	0.00427	1.45E-01	######
	-10	0.22067	-0.145435	0.72817	0.83335	-0.0088	1.37E-01	######
	-9	0.24357	-0.145239	0.72852	0.83391	0.00219	1.41E-01	######
	-8	0.23933	-0.145166	0.72866	0.83435	-0.0175	1.24E-01	######
	-7	0.22744	-0.145215	0.729	0.83452	-0.0329	9.26E-02	######
	-6	0.2224	-0.145117	0.72886	0.83455	-0.0352	5.85E-02	######
	-5	0.24917	-0.145313	0.729	0.83386	-0.0277	3.20E-02	######
	-4	0.24033	-0.145215	0.72947	0.83381	-0.0143	1.88E-02	######
	-3	0.20675	-0.145093	0.73001	0.83254	-0.0015	1.84E-02	######
	-2	0.22172	-0.145068	0.72961	0.83237	0.02538	4.49E-02	######
	-1	0.25472	-0.144702	0.72927	0.83272	0.04562	9.17E-02	######
	0	0.26608	-0.145923	0.7294	0.83352	0.00853	1.01E-01	######
	1	0.25684	-0.146216	0.72861	0.83369	-0.0604	4.21E-02	######

Batch (b)	Comments
	Macro for printing out
	the experimental data
	in the batch mode
=SET.NAME("File",GET.DOCUMENT(1))	Filename of the active file
=SET.NAME("Counter",VALUE(MID(File,7,2)))	File number
=RUN("[BOOK1.XLW]Macro!Load",FALSE)	Loading data
=RUN("[BOOK1.XLW]Macro!Print",FALSE)	Printing the chart page
=WHILE(Counter>1)	
=SET.NAME("Counter",Counter-1)	Decrementing the file number
=SET.NAME("File",REPLACE(File,7,2,TEXT(Counter,"00")))	
=OPEN(File)	Next file
=RUN("[BOOK1.XLW]Macro!Load",FALSE)	Loading data
=RUN("[BOOK1.XLW]Macro!Print",FALSE)	Printing the chart page
=NEXT()	
=RETURN()	

Load (l)	**Comments**
	Macro for loading the data
	from the data files into the
	spreadsheet
=ECHO(FALSE)	No screen update
=SET.NAME("RE",GET.DOCUMENT(1))	Ave. Rect. EMG
=SELECT("R3C1:R258C1")	
=COPY()	
=ACTIVATE("[book1.xlw]RFPE.xls")	
=SELECT("R22C2")	
=PASTE()	
=SET.NAME("AC",REPLACE(RE,12,1,"1"))	Acceleration
=OPEN(AC)	
=SELECT("R3C1:R258C1")	
=COPY()	
=ACTIVATE("[book1.xlw]RFPE.xls")	
=SELECT("R22C3")	
=PASTE()	
=SET.NAME("FO",REPLACE(RE,12,1,"2"))	Force
=OPEN(FO)	
=SELECT("R3C1:R258C1")	
=COPY()	
=ACTIVATE("[book1.xlw]RFPE.xls")	
=SELECT("R22C4")	
=PASTE()	
=SET.NAME("PO",REPLACE(RE,12,1,"3"))	Displacement
=OPEN(PO)	
=SELECT("R3C1:R258C1")	
=COPY()	
=ACTIVATE("[book1.xlw]RFPE.xls")	
=SELECT("R22C5")	
=PASTE()	
=SET.NAME("IA",REPLACE(RE,12,1,"4"))	Ave. EMG
=OPEN(IA)	
=SELECT("R3C1:R258C1")	
=COPY()	
=ACTIVATE("[book1.xlw]RFPE.xls")	
=SELECT("R22C6")	
=PASTE()	

[BOOK1.XLW]Macro

=SELECT(!$B$1)	Filename of the data file
=FORMULA(REPLACE(RE,11,2,""))	
=ACTIVATE(RE)	Closing all files
=CLOSE(FALSE)	
=ACTIVATE(AC)	
=CLOSE(FALSE)	
=ACTIVATE(FO)	
=CLOSE(FALSE)	
=ACTIVATE(PO)	
=CLOSE(FALSE)	
=ACTIVATE(IA)	
=CLOSE(FALSE)	
=ACTIVATE("[book1.xlw]RFPE.xls")	Activating the spreadsheet
=RETURN()	


counts
10
5
0
counts*ms
100
50
0
-50
100
ISIs
0
-200
-150
-100
-50
0
50
100
150
200
time (ms)
trial
10
80
60
40
20
0
presimulus discharge (ms)
0
-50
-100
-200
-150
-100
-50
0
50
100
150
200

time	Trial number	Sorted	time bins		PSTH	CUSUM			Interval
-175	1	-3	-200	0	0	-1.76617	0.416667	57	57.30337
-118	1	-3	-199	3	3	-0.53234	0.606667	62	Line
-56	1	-3	-198	1	1	-1.29851	0.813333	53	-200
-3	1	-3	-197	1	1	-2.06468	0.99	52	200
49	1	-3	-196	1	1	-2.83085	1.163333	40	0
89	1	-3	-195	3	3	-1.59701	1.296667	49	57.30337
138	1	-3	-194	0	0	-3.36318	1.46	57	
195	1	-3	-193	3	3	-2.12935	1.65	-344	
-149	2	-40	-192	2	2	-1.89552	1.503333	59	
-90	2	-40	-191	0	0	-3.66169	1.7	50	
-40	2	-40	-190	0	0	-5.42786	1.866667	51	
11	2	-40	-189	8	8	0.80597	2.036667	48	
59	2	-40	-188	0	0	-0.9602	2.196667	48	
107	2	-40	-187	1	1	-1.72637	2.356667	66	
173	2	-40	-186	1	1	-2.49254	2.576667	-324	
-151	3	-27	-185	2	2	-2.25871	2.496667	63	
-88	3	-27	-184	1	1	-3.02488	2.706667	61	
-27	3	-27	-183	3	3	-1.79104	2.91	69	
42	3	-27	-182	0	0	-3.55721	3.14	43	
85	3	-27	-181	1	1	-4.32338	3.283333	49	
134	3	-27	-180	1	1	-5.08955	3.446667	48	
182	3	-27	-179	1	1	-5.85572	3.606667	-339	
-157	4	-27	-178	1	1	-6.62189	3.476667	66	
-91	4	-27	-177	3	3	-5.38806	3.696667	64	
-27	4	-27	-176	2	2	-5.15423	3.91	45	
18	4	-27	-175	3	3	-3.9204	4.06	49	
67	4	-27	-174	0	0	-5.68657	4.223333	50	
117	4	-27	-173	1	1	-6.45274	4.39	48	
165	4	-27	-172	4	4	-4.21891	4.55	-358	
-193	5	-27	-171	2	2	-3.98507	4.356667	55	
-138	5	-27	-170	3	3	-2.75124	4.54	55	
-83	5	-27	-169	1	1	-3.51741	4.723333	56	
-27	5	-27	-168	1	1	-4.28358	4.91	66	
39	5	-27	-167	5	5	-1.04975	5.13	33	
72	5	-27	-166	0	0	-2.81592	5.24	48	
120	5	-27	-165	2	2	-2.58209	5.4	56	
176	5	-27	-164	1	1	-3.34826	5.586667	-365	
-189	6	-17	-163	4	4	-1.11443	5.37	63	
-126	6	-17	-162	1	1	-1.8806	5.58	51	
-75	6	-17	-161	2	2	-1.64677	5.75	58	
-17	6	-17	-160	2	2	-1.41294	5.943333	60	
43	6	-17	-159	1	1	-2.1791	6.143333	57	
100	6	-17	-158	0	0	-3.94527	6.333333	59	
159	6	-17	-157	2	2	-3.71144	6.53	-316	
-157	7	-17	-156	1	1	-4.47761	6.476667	51	
-106	7	-17	-155	2	2	-4.24378	6.646667	46	
-60	7	-17	-154	3	3	-3.00995	6.8	43	
-17	7	-17	-153	1	1	-3.77612	6.943333	56	
39	7	-17	-152	4	4	-1.54229	7.13	37	
76	7	-17	-151	4	4	0.69154	7.253333	56	
132	7	-17	-150	2	2	0.92537	7.44	54	
186	7	-17	-149	4	4	3.1592	7.62	-369	
-183	8	-27	-148	0	0	1.39303	7.39	54	
-129	8	-27	-147	2	2	1.62687	7.57	50	

	MU(M)	**Comments**
	=ECHO(FALSE)	No screen updating
	=SELECT("R54C1:R2053C2")	
	=CLEAR(3)	
	=SET.NAME("Counter",0)	One or more files
	=WHILE(Counter<((MU.XLW)MU.XLS!$D$1))	
	=SET.NAME("Counter",Counter+1)	
	=SET.NAME("Ad",ADDRESS(1,Counter+4,1,FALSE,"(MU.XLW)MU.XLS"))	
	=SET.NAME("FN",TEXTREF(Ad))	Filename
	=SET.NAME("FN",REPLACE(FN,LEN(FN)+1,4,"t.dat"))	
	=SET.NAME("PATH",REPLACE(!$A$2,LEN(!$A$2)-2,1,!$B$1))	Directory
	=SET.NAME("PATH",REPLACE(PATH,LEN(PATH),1,FN))	
	=OPEN(PATH,,,3)	Opening the data file
	=SELECT("R1C1:R1401C1")	
	=COPY()	Copying the data
	=ACTIVATE("(MU.XLW)MU.XLS")	
	=SELECT("R16000C1")	
	=SELECT.END(3)	
	=SET.NAME("last",SELECTION())	
	=SELECT(,"r(1)c")	
	=PASTE.SPECIAL(1,2,FALSE,FALSE)	Pasting the data
	=ACTIVATE(FN)	to the spreadsheet
	=SELECT("R1C2:R1401C2")	
	=COPY()	
	=ACTIVATE("(MU.XLW)MU.XLS")	
	=SELECT("R16000C2")	
	=SELECT.END(3)	
	=SELECT(,"r(1)c")	
	=PASTE.SPECIAL(1,2,FALSE,FALSE)	
	=COPY()	
	=ACTIVATE(FN)	
	=CLOSE()	Closing the data file
	=NEXT()	
	=ACTIVATE("(MU.XLW)MU.XLS")	
	=SELECT("R16000C1")	
	=SELECT.END(3)	
	=SET.NAME("last",SELECTION())	
	=SELECT("R54C6:R454C6")	Clearing the PSTH
	=FORMULA.ARRAY(0,SELECTION())	
	=SET.NAME("Counter",53)	**Calculating PSTH**
	=SET.NAME("Run",!$B$54)	**and sorted raster**
	=SELECT(!$F$254)	
	=WHILE(Counter<GET.CELL(2,last))	
	=SET.NAME("interval",GET.CELL(5,OFFSET(!$A$1,Counter,0)))	
	=SET.NAME("CurR",GET.CELL(5,OFFSET(!$B$1,Counter,0)))	
	=SET.NAME("reference",OFFSET((MU.XLW)MU.XLS!$F$254,interval,0))	
	=FORMULA(GET.CELL(5,reference)+1,reference)	

	=IF(CurR=Run)	
	=IF(interval<0)	
	=SET.NAME("PreSt",interval)	Pre-stimulus interval
	=END.IF()	
	=ELSE()	
	=SELECT(OFFSET(!$D$1,Counter-1,0))	
	=FORMULA(PreSt,SELECTION())	
	=WHILE(Run=GET.CELL(5,OFFSET(SELECTION(),-1,-2)))	
	=SELECT(,"R(-1)C")	
	=FORMULA(PreSt,SELECTION())	
	=NEXT()	
	=SET.NAME("PreSt",-200)	
	=IF(interval<0)	
	=SET.NAME("PreSt",interval)	
	=END.IF()	
	=SET.NAME("Run",CurR)	
	=END.IF()	
	=SET.NAME("Counter",Counter+1)	
	=NEXT()	
	=SELECT(OFFSET(!$D$1,Counter,0))	
	=WHILE(Run=GET.CELL(5,OFFSET(SELECTION(),-1,-2)))	
	=SELECT(,"R(-1)C")	
	=FORMULA(PreSt,SELECTION())	
	=NEXT()	
	=SELECT("R54C6:R454C6")	
	=COPY()	
	=SELECT("R54C7:R454C7")	
	=PASTE()	
	=SELECT((MU.XLW)MU.XLS!$E$1)	
	=PRINT(2,1,1,1,FALSE,FALSE,1,FALSE,1)	Printing page 1
	=RETURN()	(the charts)

APPENDIX B

CURRICULUM VITAE

ANDREW VICTOR POLIAKOV
CURRICULUM VITAE

April 16, 1994

PRESENT POSITION: Ph. D. student, Department of Physiology, University of Adelaide.

PERSONAL

Date/Place of Birth: March 18, 1964, U.S.S.R.
Citizenship: Russian Federation
Family Status: Married to Svetlana, one child

Phone:
Fa
Email

ADDRESS

Work: Dept. of Physiology, University of Adelaide, Adelaide, SA 5005, Australia.
Home: 3

EDUCATION

Moscow Institute of Physics and Technology Dolgoprudni, Moscow Area, U.S.S.R.	Diploma of Engineer-Physicist-Researcher (equivalent to M.Sc. in Biophysics, supervisor Prof. Gurfinkel V.S.)	1980-87
Dept. of Physiology, University of Adelaide, Adelaide, Australia	Ph.D. student (supervisor Assoc. Prof. Miles T.S.)	1991- present

PROFESSIONAL EXPERIENCE

Laboratory for Motor Control Institute for Problems of Information Transmission Academy of Science, Moscow, U.S.S.R..	Engineer (Part time)	1986-87
Laboratory for Motor Control Institute for Problems of Information Transmission, Academy of Science, Moscow, U.S.S.R..	Research Fellow in Training	1987-88
Laboratory for Motor Control Institute for Problems of Information Transmission, Academy of Science, Moscow, U.S.S.R..	Junior Research Fellow	1988-91
Department of Physiology University of Adelaide, Adelaide, Australia	Ph.D. student	1991- present

MAJOR FIELDS OF RESEARCH

During the period of my training at the Moscow Institute of Physics and Technology at the Department of Physics in Alive Systems (Biophysics) I studied the effects of muscle prehistory on isometric muscle contraction in humans, evoked by electrical stimulation of the motor point. This included various aspects of the phenomenon of potentiation of muscular contraction during activation.

My work at the Institute for Problems of Information Transmission concerned a number of projects related to muscle contractile properties and electromyography. In particular, I investigated the contractile properties of fast and slow twitch fibres in human soleus muscles by activating different populations of fibres evoking either M-response or H-reflex in the muscle. Long-term post-activation alterations of muscle contractile properties were revealed to alter significantly the pattern of unfused tetanus in human flexor digitorum brevis. I also examined the surface EMG pattern in soleus muscle subjected to vibration, and compared the experimental results with a computer model I developed. The effect of synaptic noise on the cross-correlation of motor neuron discharges to stimuli was studied theoretically, using random process theory.

My research at the University of Adelaide is an investigation of the stretch reflex in single motoneurons in the human masticatory system. I have also pursued my interest in some theoretical aspects of motoneuron firing patterns in humans and animals, in particular in relation to the problem of an appropriate description of their random properties, and have analysed several methodological and theoretical aspects of stretch reflex studies and EMG data treatment.

HISTORY OF SUPPORT

Ph.D. studies:
Overseas Postgraduate Research Scholarship and University of Adelaide Scholarship 1991-94

TEACHING EXPERIENCE

University of Adelaide, Adelaide

Demonstrator, Membrane Physiology, Part of Cellular Physiology Subject	3 rd year Science students	1992, 93
Demonstrator in Neurophysiology (introduced new experimental project)	3 rd year Science students	1991, 92, 93

PRESENTATIONS

Presentations at National and International meetings.

Meeting on Motor Control
Leningrad, 1989

Australian Physiological and Pharmacological Society
Melbourne, 1991, Sydney, 1992

Australian Neuroscience Society
Adelaide, 1992

Society for Neuroscience (USA)
Washington DC, 1993

Invited Seminars at Other Universities and Research Institutions

Department of Physiology, Monash University, Melbourne, Australia, June 29, 1993.
Department of Physiology and Biophysics, Washington University, Seattle, U.S.A., November 18, 1993.
Division for Neuroscience, University of Alberta, Canada, November 21, 1993.

Presentations at Local Meetings

Motor Control Group Seminars (15-20 participants) Institute for Problems of Information Transmission, Moscow	1888, 90
Conferences of Young Scientists, Institute for Problems of Information Transmission, Moscow	1988, 89
Department of Physiology Seminar The University of Adelaide, Adelaide	1992, 93

OTHER PROFESSIONAL ACTIVITIES AND RECOGNITIONS

Awards:

PhD studentship: Overseas Postgraduate Research Scholarship and University of Adelaide Scholarship

Post-doctoral training:

Alberta Heritage Foundation for Medical Research (CANADA), Full Time Fellowship (declined)	1993
Spinal Cord Research Foundation (USA), SCRF Grant #1314 (declined)	1993
International Human Frontier Science Program Organization, Long-term Fellowship	1994

Membership of Professional Societies:

Australian Physiological and Pharmacological Society

Travel Grants:

From the Academy of Science, U.S.S.R., Grant to attend the International Meeting on Motor Control, (1990), Warsaw, Poland.

From the International Brain Research Organisation, Travel grant for young scientists (declined). To attend the Third IBRO World Congress of Neuroscience, (1991), Montreal, Canada.

From the Australian Physiological and Pharmacological Society, Travel grants. To attend the APPS meetings, Melbourne, (1991), Sydney, (1992).

PUBLICATIONS

THESIS TITLES

1. Potentiation of muscular contraction. (MPhTI, Moscow, 1987)

2. Stretch reflex in human masticatory muscles (The University of Adelaide, Adelaide, submitted)

A. MANUSCRIPTS PUBLISHED IN REFEREED JOURNALS

A1. Polyakov A.V. 1987. Electromyogramme-Force Relationship during Potentiation. **Bulletin of Experimental Biology and Medicine** 104: 521-523. (in Russian, translated into English by Consultant Bureau, Plenum Publishing, NY, 1988, 1496-1498).

A2. Gurfinkel V.S., Levick Ju.S., Polyakov A.V. 1988. The Twitch Mechanical Alteration in Human Skeletal Muscle under Different Rates of Activation. **Human Physiology** 14: 1001-1007. (in Russian).

A3. Gurfinkel V.S., Levick Ju.S., Polyakov A.V. 1989. Contractile Properties of Fast and Slow Twitch Fibres in Human Calf Muscles. **Human Physiology** 15: 85-89. (in Russian, translated into English by Consultant Bureau, Plenum Publishing, NY, 1990, 369-373).

A4. Gurfinkel V.S., Levick Ju.S., Polyakov A.V. 1990. Long-term Post activation Contraction Changes in Human Skeletal Muscle. **Human Physiology** 16: 71-76. (in Russian, translated into English by Consultant Bureau, Plenum Publishing, NY, 1991, NY).

A5. Lebedev M.A. and Polyakov A.V. 1991. The Analysis of Human Soleus Muscle Surface EMG in Conditions of Vibrational Stimulation. **Neurophysiology, Kiev** 23: 57-65. (in Russian, translated into English by Consultant Bureau, Plenum Publishing, NY, 1992, 47-54).

A6. Polyakov A. V. 1991. Synaptic Noise and the Cross-correlation of Motor neuron discharges and stimuli. **Neuroreport** 2: 489-492.

A7. Lebedev M.A. and Polyakov A.V. 1992. Analysis of surface EMG of human soleus muscle subjected to vibration. **Journal of Electromyography and Kinesiology** 2: 26-35.

A8. Poliakov, A.V. and Miles, T.S. 1992. Quantitative analysis of reflex responses in the averaged surface electromyogram. **Journal of Neuroscience Methods,** 43: 195-200.

A9. Miles, T.S. Poliakov, A.V. and Flavel S.C. 1993. An apparatus for controlled stretches of human jaw-closing muscles. **Journal of Neuroscience Methods,** 46: 197-202.

A10. Poliakov, A.V. and, Miles, T.S. 1994. Stretch reflexes in human jaw muscles. **Journal of Physiology,** 476: 323-331.

A11. Poliakov, A.V., Miles, T.S. and Nordstrom, M.A. 1994. A new approach to the estimation of post-synaptic potentials in human motoneurons. **Journal of Neuroscience Methods,** in the press.

A12. Miles, T.S., Poliakov, A.V. and Nordstrom, M.A. Responses of Human Masseter Motor Units to Stretch. **Journal of Physiology,** accepted for publication (with revisions).

A13. Poliakov, A.V., Miles, T.S and Nordstrom, M.A. Discharge Patterns of Tonically Firing Human Motoneurons. Submitted .

B: PUBLISHED ABSTRACTS AND SHORT COMMUNICATIONS

B1. Polyakov A.V. 1988. Contractile Properties of Fast and Slow Twitch Fibres in Human Calf Muscles. **Information Transmission and Treatment Systems. Part 2. Institute for Problems of Information Transmission, USSR Academy of Science. Moscow**, 75-78. (in Russian)

B2. Lebedev M.A. and Polyakov A.V. 1989. Switched Muscle Activity Induced with Vibration. 1. Surface EMG spectral analysis. **Information Transmission and Treatment Methods. Institute for Problems of Information Transmission, USSR Academy of Science. Moscow**, 54-58. (in Russian)

B3. Polyakov A.V. and Lebedev M.A. 1989. Switched Muscle Activity Induced with Vibration. 2. Motor Unit discharge. **Information Transmission and Treatment Methods. Institute for Problems of Information Transmission USSR Academy of Science. Moscow**, 59-61. (in Russian)

B4. Polyakov A.V. 1991. The effect of synaptic noise on the cross-correlation of motoneuron discharges to stimuli. **Proc. Aust. Physiol. Pharmacol. Soc.** 22: 154P

B5. Poliakov A.V. and Miles T.S., 1992. Stretch reflex in human masseter. **Proc. Aust. Neurosci. Soc.** 3: 91P

B6. Poliakov A.V. and Miles T.S. 1992. A new approach to the quantitative analysis of reflex responses in the averaged surface electromyogram. **Proc. Aust. Physiol. Pharmacol. Soc.** 23: 233P

B7. Poliakov A.V., Miles T.S. and Nordstrom M.A 1993. A single motor unit analysis of the mechanism for force generation in stretch reflexes. **Proc. Aust. Physiol. Pharmacol. Soc.** 24: 133P.

B8. Poliakov A.V., Miles T.S. and Nordstrom M.A 1993. Reflex responses of human masseter to stretch. **Soc. Neurosci. Abstr.** 19: 61.5

APPENDIX C

Reprints of Published Papers Associated with this Thesis

Poliakov, A.V., and Miles, T.S., (1992) Quantitative analysis of reflex responses in the averaged surface electromyogram.
Journal of Neuroscience Methods, v. 43 (2-3), pp. 195-200.

Miles, T.S., Poliakov, A.V., and Flavel, S.C., (1993) An apparatus for controlled stretch of human jaw-closing muscles.
Journal of Neuroscience Methods, v. 46 (3), pp. 197-202.

Poliakov, A.V., and Miles, T.S., (1994) Stretch reflexes in human masseter.
Journal of Physiology, v. 476 (2), pp. 323-331.