FUNCTIONAL CHARACTERISTICS OF MOTOR UNITS IN HUMAN MASSETER.

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by

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ABSTRACT

The functional characteristics of human masseter motor units were studied during voluntary isometric contractions. The primary goal was to quantify fatigue of single motor units in human masseter during a prolonged voluntary isometric contraction. This was accomplished using the spike – triggered averaging (STA) technique. The human masseter was found to be comprised predominantly of fast – twitch units with a broad spectrum of fatiguability, and very few physiological type S motor units. There was also a poor correlation between fatiguability and motor unit twitch tension or contractile speed. Previous studies have shown the human masseter to have rather unique histological features, and the new physiological data facilitate interpretation of the possible functional significance of this muscle's peculiar anatomical and histochemical properties.

During the fatigue test the contribution of the motor unit to the surface electromyogram (EMG) was assessed with STA, and was found to vary systematically with time. Under the conditions of this study the changes in the motor unit contribution to the surface EMG did not correlate well with contractile fatigue.

In the course of this work, a close examination of the use and limitations of STA was performed. Motor unit synchronization in the masseter was assessed by cross – correlation of unit firing times, and found to be weak, but widespread. This degree of synchrony was considered to have only a minor effect on measures obtained by STA in the masseter. A systematic investigation of the effect of the motor unit firing pattern on the STA twitch was performed, with the aid of a computer averaging program which allowed precise control over the spikes accepted as triggers on the basis of their pre – and post – trigger firing intervals. These findings helped to improve the accuracy of the twitch fatigue study.

Several features of the control of masseter motor units were also investigated. The activation force threshold of the motor units was found to vary in a consistent way with muscle length, so that at longer muscle lengths the threshold force increased. It was shown that the change in activation threshold was closely related to the change in passive tension at different muscle lengths. The variability of motor unit discharge increased following prolonged activity, and the initial discharge variability was found to correlate with fatiguability of the motor unit. This represents a link between the activation pattern of the motoneurone and the muscle fibres it innervates. Observation of the relative mean firing rates of concurrently – active units during prolonged activation revealed that the initial size – structured hierarchy of activity was not preserved throughout the contraction.

DECLARATION

I declare that this thesis is a record of original work and that it contains no material which has been accepted for the award of any other degree or diploma in any University.

To the best of my knowledge and belief, this thesis contains no material previously published or written by any other person, except where due reference is given in the text of the thesis.

I consent to this thesis being made available for photocopying or loan.

Michael A. Nordstrom.

November, 1988.

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AIMS AND GENERAL INTRODUCTION

The masseter is one of the major muscles of mastication, yet the physiological properties of its motor units have not been widely studied. In particular, single unit fatigue data for the masseter is not available from either animals or humans. Whole – muscle fatigue data is difficult to interpret without this information, and much of the available whole – muscle fatigue data for the masseter has not provided objective measures of fatigue. A major aim of this thesis was to quantify fatigue of single motor units in the human masseter during a voluntary contraction, and to perform a physiological classification of the motor units, using the spike – triggered averaging (STA) technique. The unique histological features of the human masseter make it a particularly interesting, and instructive, muscle for the study. These results are presented in Chapter 5.

A second aim was to examine at the motor unit level, the relationship between electrical and mechanical events occurring during fatigue. This approach has the advantage that fatigue can be objectively measured in terms of loss of force, and therefore fatigue can be directly compared with changes in the motor unit EMG signal. This helps to establish the causes of force loss, and also indicates how well the EMG signal may reflect contractile fatigue under these conditions. This issue is addressed in Chapter 6.

The realization of these goals in voluntary contractions in humans was dependent on the STA technique. Although it has been widely – used for measuring the mechanical properties of motor units, several criticisms of this technique have been raised in the past. There has been concern over the extent of fusion of the STA twitches, and also the possibility of large errors due to motor unit synchronization. For these reasons, considerable effort was devoted to evaluating the accuracy and limitations of the STA technique for the planned investigations. The extent of motor unit synchronization in the masseter was assessed in Chapter 2, and the implications of these findings for the use of the STA technique in the masseter were considered. In Chapter 3, the effect of motor unit firing patterns on the STA twitch was investigated, along with an evaluation of the implications for fatigue – testing in the masseter. The desire for optimal temporal resolution of the twitch measurements for the fatigue tests led to efforts to improve the STA technique. A means of improving the signal – to – noise ratio, and hence the reliability of the STA twitches, is described in Chapter 4.

Several aspects of the control of motor unit activity were also investigated. The effects of a change in muscle length on the motor unit recruitment threshold were studied in Chapter 7. In Chapter 8, the variability of motor unit discharge in the

masseter was measured, and the effects of the duration of the contraction and fatigue on this parameter assessed. In addition, correlations were sought between motor unit physiological properties (determined by STA) and discharge variability, as this gives an insight into the link between motoneurone and muscle unit properties. A model is presented which clarifies the relationship between noise in the membrane potential depolarization trajectory and interspike interval variability.

It is known that motor unit recruitment order is relatively stable under most normal conditions. It is not known how well this order is preserved in motor unit firing patterns during prolonged activation. The final aim was to establish whether motor unit activation patterns may be altered from their original size – structured relationships during normal voluntary activation of the muscle. This was achieved by observing the pattern of changes in the mean firing rates of active motor units during a prolonged contraction, and is described in Chapter 9.

CHAPTER 1

LITERATURE REVIEW

1.1 The motor unit concept.

The concept of the motor unit was introduced in a paper by Liddell & Sherrington in 1925, in which it was described as "the motoneurone axone and its adjunct muscle – fibres". The modern definition includes the motoneuronal cell body as part of the motor unit. The acceptance of the motor unit as the smallest functional unit of skeletal muscle is based on several lines of evidence:

i) With few exceptions (Emonet – Dénand *et al.*, 1971; Crandall *et al.*, 1981), the muscle fibres of a motor unit lie within a single anatomically – defined muscle (Stuart & Enoka, 1984).

ii) There is a 1:1 relationship between the discharge of a motoneurone and all the muscle fibres of the motor unit. This appears to be the case under normal conditions in vivo with low (physiological) rates of activation (Bigland – Ritchie *et al.*, 1979), although with higher rates of stimulation neuromuscular transmission may fail in vivo (Naess & Storm – Mathisen, 1955; Bigland – Ritchie *et al.*, 1979). It may also fail in vitro even with low rates of stimulation (Krnjevic & Miledi, 1958).

iii) In mature muscles each muscle fibre is innervated by only one motoneurone (Bagust *et al.*, 1973; Brown & Matthews, 1960; Feindel *et al.*, 1952). Exceptions to this rule can be found in the muscles of new – born cats and rats (Bagust *et al.*, 1973; Brown *et al.*, 1976).

iv) Finally, there is the observation that all the muscle fibres within the motor unit have a very similar metabolic profile, in contrast to the wide range found in randomly selected fibres within the same muscle (Edström & Kugelberg, 1968; Burke *et al.*, 1971; Burke *et al.*, 1973; Nemeth *et al.*, 1981; Nemeth *et al.*, 1986).

With this principle of the motor unit as the functional quantum used in the production of force by a muscle, study of muscle function at the level of the motor units has contributed a great deal in recent years to the understanding of how muscles perform their tasks.

1.2 Muscle fibre types and motor unit types

The existence of slowly – contracting "red" and rapidly – contracting "white" muscle suggested that there should be at least two types of motor units with different properties. The demonstration by Gordon and Phillips in 1953 of slow – twitch components in the nominally fast – twitch tibialis anterior of the cat, suggested that muscles may be composed of motor units with different characteristics. By the early 1960's histochemical techniques had made it possible to distinguish a variety of different fibre types within a muscle (Dubowitz & Pearse 1960; Romanul, 1964; Stein & Padykula, 1962). Since this time a number of histochemical and biochemical methods for detecting more subtle differences between muscle fibres have been developed, and subsequent investigations have shown that most muscles are fairly heterogenous in nature, being comprised of muscle fibres with a range of properties (Dubowitz & Brooke, 1973).

The main differences in the various muscle fibres that can be revealed by histochemical methods are; structural proteins (myosin ATPase (MATPase) activity); metabolic enzyme systems (mitochondrial oxidative enzymes such as succinic dehydrogenase and NADH – dehydrogenase; enzymes linked to anaerobic glycolysis such as α – glycerophosphate dehydrogenase); and the relative amounts of substrates such as glycogen or fat. Two main fibre classification schemes based on histochemical methods have evolved, and with each the fibres of most muscles can be broadly divided into three categories. The scheme of Brooke & Kaiser (1970) is based primarily on myosin ATP – ase reactions (types I, IIA, IIB, IIC); while that of Peter *et al.* (1972) also uses staining for metabolic enzymes (FG, fast – twitch, glycolytic; FOG, fast – twitch, oxidative and glycolytic; SO, slow – twitch, oxidative).

A major advance in the study of the organisation of muscle came with the demonstration that the different fibre types were related to different motor units in the muscles. This had been suspected, but it was the advent of the glycogen depletion technique which allowed direct comparison of motor unit and muscle fibre properties (Edström & Kugelberg, 1968). With this technique, repetitive stimulation of one motor unit in the muscle sufficient to deplete the fibres of that unit of glycogen, is combined with serial cross – sectional staining to determine the histochemical profile of the muscle fibres of the unit. All fibres of a given motor unit were shown to have similar histochemical properties, which meant that differences among the fibres in a muscle were the result of differences in the properties of the muscle fibres of different motor units (Edström & Kugelberg,

1968). The metabolic uniformity of the muscle fibres of a motor unit has been disputed in recent years (Roy *et al.*, 1984). This is an important question, as it bears on the whole concept of regarding the motor unit as a functional entity. Recent studies using quantitative microanalytical biochemistry (as opposed to qualitative histochemistry) have shown that muscle fibres of a motor unit are very homogeneous with respect to the activity of a number of metabolic enzymes, and reaffirm the status of the motor unit as a functional quantum (Nemeth *et al.*, 1986). Because of this homogeneity of the fibres of a single motor unit, it is sometimes convenient to use the term "muscle unit" (Burke, 1967) when the properties of the muscle fibres of a motor unit are considered.

Consideration of physiological properties such as the time to peak twitch tension, peak tetanic tension, and conduction velocity of the motor axon also reveals differences between motor units. The first systematic description of the physiological properties of the motor units within an individual muscle was that of Bessou *et al.* (1963) in the first deep lumbrical of the cat. This was followed shortly after by extensive investigations by Henneman and colleagues in the cat medial gastrocnemius (Wuerker *et al.*, 1965) and soleus (McPhedran *et al.*, 1965) in which it was clear that muscles, even a relatively histochemically homogeneous one such as cat soleus, contain motor units with a wide range of physiological properties. Henneman and Olson (1965) suggested that the diversity of these properties must be related to histochemical differences between the fibres although there was no direct evidence for this at that time. The pioneering glycogen depletion study by Edström & Kugelberg (1968) was the breakthrough needed to test this hypothesis, by allowing a direct comparison of motor unit physiological properties and the histochemistry of its constituent muscle fibres. In this initial study in the anterior tibial muscle of the rat, there was a poor correlation between motor unit contraction time and fibre type (all units were fast – twitch). There was, however, a good correlation between the decline in mechanical output (fatiguability) of the unit with continuous low – frequency stimulation and the unit's histochemical fibre type. Burke and colleagues combined physiological tests with the glycogen depletion technique to study motor units of cat medial gastrocnemius muscle and were able to correlate the physiological properties of the units with their histochemical profiles (Burke *et al.* 1971; Burke *et al.*, 1973). The physiological tests used to classify the units involved measurement of the time to peak tension of the twitch, combined with a "sag" test to separate fast and slow motor units. "Sag" is the tendency for the unfused tetanic response to decline in peak tension with time, and is claimed to occur only in fast units (Burke *et al.*,

1971), although this has been subsequently disputed (Reinking *et al.*, 1975). A fatigue test was also applied to the units which consisted of a train of stimulus pulses at 40 Hz for 330 ms, repeated each second for 2 minutes. With this test the slow units are very resistant to fatigue, and the fast population can be separated into 3 groups with varying resistance to fatigue: FF, fast, fatiguable; FI, fast, intermediate fatigue resistance; FR, fast, fatigue resistant. This combination of physiological tests, occasionally with minor variations, has become a standard for categorising motor unit populations in subsequent studies (see below).

The physiological properties of motor units are associated with two main histochemical features of their muscle fibres. The first is the degree of staining for MATPase, which has been shown to be proportional to contractile speed of muscle units in the rat soleus (Kugelberg, 1973;1976), and this property can be used as a general but not exact guide to the contractile speed of motor units in most muscles (Burke, 1981*a*). However, Burke (1981*a*) has pointed out that although the degree of MATPase activity is an important indicator of contractile speed, it is not the only factor which determines the contractile speed of a muscle, and that other factors are also involved, such as processes involved in excitation – contraction coupling, previous activation history, fibre length, and the characteristics of the series elastic element. These factors may explain the fact that in the cat medial gastrocnemius the contraction times of the fast – twitch units vary over at least a two – fold range without corresponding gradations in MATPase staining. Furthermore, the range of contraction times of the type S units in this muscle nearly overlaps that of the type FR units despite the difference in their MATPase staining (Burke *et al.*, 1973). Also, Edström & Kugelberg (1968) found no correlation between MATPase staining and contractile speed in the fast – twitch tibialis anterior. These findings emphasise that absolute values of contractile speed of the units cannot be inferred from the staining for this enzyme, and that a clear distinction between fast and slow units on the basis of staining for MATPase does not necessarily indicate a bimodal distribution of unit twitch – contraction times. Nevertheless, as a general rule, slow – twitch units usually have low MATPase activity (type I fibres) and fast – twitch units usually have high MATPase activity (type II fibres).

The second association between motor unit physiological and histochemical properties is in the staining for metabolic enzymes. The staining for oxidative enzymes has been shown to correlate directly with unit fatigue resistance (Edström & Kugelberg, 1968; Burke *et al.*, 1973; Kugelberg & Lindegren, 1979) and ranges from low in the fibres of FF units (corresponding to type IIB), to moderate in

the F(Int) and moderate to high in the FR units (type IIA) and high in the S units (type I) (Burke *et al.*, 1971; Burke *et al.*, 1973). The advent of microanalytical biochemical techniques has allowed more quantitative assessment of these relationships than is possible using glycogen depletion/histochemistry alone. Hamm *et al.* (1988) have shown that motor units of the same physiological type in cat tibialis posterior have very similar activities for a range of metabolic enzymes, and that the population could be sub – divided into identical groups using either physiological or biochemical properties.

Consequently, the animal evidence suggests that motor units may be categorised using either histochemical, biochemical or physiological criteria, and that classification by either method results in equivalent sub – groups of motor units (Burke, 1981*a*; Hamm *et al.*, 1988). There is a fairly clear delineation between fast and slow motor units using these criteria. Slow motor units (type S units) are characterised by long contraction times, low peak tetanic tensions, high resistance to fatigue, absence of sag, high levels of oxidative enzymes, low levels of anaerobic enzymes, and low levels of staining for MATPase (Stuart & Enoka, 1984). Further evidence for a clear distinction between fast and slow units is the fact that slow myosin is antigenically distinct from fast myosin (Gauthier *et al.*, 1978). The type S units correspond to the type I or SO fibre type classification.

The differences among the fast motor unit population are not as distinct. Fast motor units can be divided into three subgroups, based on their resistance to fatigue, using the fatigue test of Burke *et al.* (1973). With this test the highly fatiguable units are classified FF, and also tend to have the highest peak tetanic tensions, and low levels of oxidative enzymes (fibre type IIB or FG). The fast units with the lowest fatiguability are classified FR, and have high levels of oxidative enzymes (fibre type IIA or FOG). Units of intermediate fatiguability are classified F(Int), stain moderately for oxidative enzymes, and have moderate to high tetanic tensions (reviewed by Stuart & Enoka, 1984). This unit type is found only in small numbers in most muscles and it has been suggested that it represents an intermediate form in the process of conversion from FF to FR as a result of training or usage demands on the muscle (Jansson *et al.*, 1978).

The many studies in a range of muscles in the cat and other species such as the rat have led to a consensus that in general the motor unit types conform to the basic divisions elaborated above (reviewed in Burke, 1981*a*; Stuart & Enoka, 1984). However, it is prudent to regard each muscle as unique, and to remember that the differences between motor unit types are relative rather than absolute,

and that in many muscles there is a great deal of overlap in the properties of the motor units, with no clear boundaries between motor unit types. There can be differences in properties of muscle units of the same functional type in different muscles in the same species (e.g., the histochemical profile of type I units in cat soleus and medial gastrocnemius; Burke & Tsairis, 1974), and large differences in the same muscles in different species (e.g., rat and cat muscle histochemistry; Kugelberg, 1973). Individuals may also have significant differences dependent on such variables as sex (Brooke & Engel, 1969) and the extent of training (Jansson & Kaijser, 1977; Saltin *et al.*, 1977), which are particularly evident in human muscle.

1.3 Human motor unit types.

1.3.1 Histochemistry.

Human skeletal muscle also contains the two main fibre types, types I and II, based on staining for MATPase (Padykula & Herman, 1955). Brooke and Kaiser (1970) showed that the type II fibres in human muscle contained the same subgroups (types IIA, IIB, and IIC) found in animal muscles. The various muscles in the body differ in the relative proportions of the fibre types. In general, most muscles have a mean fibre composition of about 50% type I fibres, but some muscles such as soleus may have close to 100% type I fibres in some individuals, while triceps brachii may have less than 20% type I (Johnson *et al.*, 1973). In humans there is also a particularly wide variation in fibre type proportions in the same muscle in different individuals (Johnson *et al.*, 1973). For example, in one study, the vastus lateralis was biopsied and showed an average fibre composition of 52.2% type I fibres, but with a range of 13 – 98% (Gollnick *et al.*, 1972). This diversity may be the result of genetic predetermination (Komi *et al.*, 1977), or the effects of use or training (Jansson *et al.*, 1978). There is still controversy as to whether type I and II fibre type interconversion is possible in humans, with evidence for (Jansson *et al.*, 1978) and against (Saltin *et al.*, 1977; Saltin *et al.*, 1976). It does seem likely that the subdivisions of type II fibres are interconvertible with training (Andersen & Henriksson, 1977; Jansson & Kaijser, 1977).

1.3.2 Physiological Properties.

Despite the obvious importance of studying the physiological properties of human motor units, there have been very few studies which have addressed this question.

Buchtal & Schmalbruch (1970) were the first to record contraction times and forces of small fibre bundles in a number of human muscles, using controlled intramuscular microstimulation. They were able to demonstrate that human muscles contain fibre bundles with a range of contractile speeds, and that the distribution of contractile speeds correlated with the histochemical evidence available for these muscles. In this study the properties of some single motor units were determined by spike – triggered averaging (STA), and the contraction times of the motor units also showed a range similar to that of the small fibre bundles. Sica & McComas (1971) studied motor unit properties in extensor hallucis brevis using graded percutaneous stimulation of motor nerves. They measured isometric twitch tensions and contraction times and reported a range of contraction times of 35 – 98 ms. An important observation was that there was no correlation between twitch tensions and contraction times in the motor units studied, in contrast to the normal situation in the cat hind – limb muscles with a heterogenous distribution of motor unit contractile speeds (Burke *et al.*, 1973; Wuerker *et al.*, 1965). Burke, Skuse & Lethlean (1974) studied abductor digiti minimi in man, and recorded the activity of a number of low – threshold units using percutaneous stimulation and ensemble averaging. They found a unimodal distribution of contractile speeds, and deduced that the motor unit population of this muscle contained type FF and FR units, but no evidence of type S units.

A major advance in human motor unit studies was the use of the STA technique by Milner – Brown *et al.* (1973*a*,*b*) to systematically study the mechanical properties of human motor units activated voluntarily in human first dorsal interosseous muscle (FDI). They reported a unimodal distribution of motor unit contraction times (30 – 105 ms) and a negative correlation between twitch tension and contractile speed. An important finding was that the motor units were recruited in order of increasing twitch tension during a voluntary contraction, which was further support for the size principle of recruitment of Henneman and colleagues (see Section 1.6.1).

Monster & Chan (1977) used the STA technique to study the motor units of extensor digitorum communis (EDC) in man. They reported that all units they tested in EDC were fast – twitch, although histochemical evidence shows approximately 50% of the fibres in this muscle are type I (Johnson *et al.*, 1973). The same conclusion was reached by Burke, Skuse & Lethlean (1974) using STA in human abductor digiti minimi. These anomalies have been generally regarded as being related to deficiencies of the STA technique itself, which tends to underestimate twitch contractile speed due to partial fusion of the twitches.

However, the possibility remains that the correlation between physiological and histochemical properties of motor units may not be as strong in human muscle as it is in animals. The aforementioned human motor unit studies using STA have not provided strong evidence on this question because they did not assess fatigue in the units. A fatigue test is essential, in addition to measuring properties such as tension and contractile speed, in order to classify motor units into physiological types. Only then can comparison be made with the physiological profiles in animal muscles, and with human muscle histochemistry.

Although the studies of the early 1970's showed that it was feasible to measure the contractile properties of single motor units in man, only three reports are found in the literature where the fatigue properties of the units have been systematically measured. Garnett *et al.* (1978) studied the motor units of human medial gastrocnemius muscle with controlled intramuscular microstimulation, which has the advantage that the glycogen depletion method may also be used, which when combined with muscle biopsy, allows direct comparison of motor unit physiological and histochemical properties. This is the only study in humans in which a direct comparison has been made between motor unit physiological and histochemical properties. They reported a bi-modal distribution of contraction times (range 40-110 ms), and units with a range of fatiguability to a stimulus paradigm of pulses of 10-20 Hz for 0.5 s, repeated once per second, or once per two seconds. The physiological and histochemical profile of the units matched reasonably well, and they deduced that the organisation of the human medial gastrocnemius was similar to that of the cat muscle, in which Burke *et al.* (1973) had demonstrated a tri-partite classification (S, FF, FR) and a correlation between motor unit physiological and histochemical properties.

Using an intramuscular stimulation technique, Young & Mayer (1981) investigated the motor units of human FDI and found a unimodal distribution of contraction times (range 34-140 ms) but no correlation between twitch tension and contractile speed (unlike the findings of Milner-Brown *et al.* (1973*b*) and Stephens & Usherwood (1977) in the same muscle using STA). The units were classified into three groups based on their contractile speed and fatiguability. Although a direct comparison was not presented, it would appear that there was not a strong correlation between twitch tension and fatiguability in the units studied.

The only previous study to investigate the fatigue properties of human motor units during a voluntary contraction was that of Stephens & Usherwood (1977), who

used the STA technique to measure the fatigue properties of motor units in FDI. They found a negative correlation between twitch tension and contraction time, and larger and faster units were more fatiguable following 5 minutes of continuous activity at 10 Hz. It was concluded that the motor units of FDI conformed to a grouping into the S, FF, and FR types found in animals, and that in voluntary contractions of this muscle the motor units were recruited in order of a decreasing resistance to fatigue.

It is evident that there is not a great deal of information regarding fatigue of motor units during a voluntary contraction in either humans or animals. Questions that remain to be answered include, firstly; how universal are the relationships between motor unit size, contractile speed and fatiguability, particularly in human muscles? In muscles in which there is a broad spectrum of motor unit types there appears to be a good correlation between these properties, but it has been suggested that this relationship relies on the existence of separate populations of fast and slow units within the muscle, and that examination of the groups separately weakens or eliminates the correlation (Goslow *et al.*, 1977). Secondly, the question of the correlation between motor unit physiological and histochemical properties in humans requires clarification. Of particular interest are muscles with a predominance of physiological fast – twitch motor units which do not appear consistent with the histochemical evidence of substantial proportions of type I fibres. Assessment of motor unit fatigue and hence physiological types in these muscles, would help to clarify this issue. The motor units of human masseter muscle, which appear to be mainly fast – twitch (Yemm, 1977*b*; Goldberg & Derfler, 1977), in a muscle with a large population of type I fibres (Eriksson & Thornell, 1983) provide one such opportunity.

1.4 Motor units in the masseter muscle.

1.4.1 Animal Data.

There have been no studies in which electrical stimulation has been used to determine the mechanical properties of jaw – muscle motor units in animals. This is presumably because of the difficulty in gaining access to the motor nerves without damaging the muscles. Consequently, the physiological properties of motor units in the jaw muscles of animals have mostly been inferred from whole – muscle studies. The consensus of the few animal studies which have appeared in the literature is that the jaw muscles are fast – twitch muscles. Most

animal studies have used direct stimulation of the muscle because of the difficulty of access to the motor nerves. Tamari *et al.* (1973) reported that the masseter of the cat was slightly faster than the temporalis. Fast contraction times have also been reported in the masseter of the rat (Nordström & Yemm, 1974) and possum (Thexton & Hiiemae 1975). The most extensive study is that of Taylor *et al.* (1973), who stimulated strips of cat masseter and temporalis and found them to be very fast – contracting, with a time – to – peak tension of around 11 – 13 ms. In this study investigation of the mechanical properties of the muscle strips was combined with histochemical analysis, and this is the only study known to the author where a fatigue test has been applied to the masseter by controlled stimulation. The fatigue test used was a train of pulses at 55 – 90 Hz, of 330 ms duration, repeated once every second. With this stimulation pattern there was a rapid loss of tension in the first minute to about 25% of the initial level, and then a gradual decline over the next 2 – 3 min. Although this test paradigm was chosen to be compatible with the standardised test of Burke *et al.* (1971) it is possible that with these high rates of stimulation fatigue resulted primarily from failure of activation of the muscle fibres, although it is not possible to assess this as muscle EMG was not recorded (see section 1.5.3). The predominant fibre type was large, with strong staining for MATPase, and weak staining for the oxidative enzyme succinate dehydrogenase (SDH). The second fibre type was of intermediate size, similar staining for MATPase, and strong staining for SDH. These types were assumed to correspond to motor unit types FF and FR, respectively, and together in the cat masseter they comprised 90% of the muscle cross – sectional area. The remaining fibres had low myosin – ATPase activity, and strong staining for SDH. These fibres corresponded to the profile of type S units.

In contrast to the situation in cats, histochemical data is available for the rhesus monkey which shows that the anterior masseter is composed of 80 – 90% type I fibres (Maxwell *et al.*, 1979), whereas the posterior masseter had a more even distribution between the three main fibre types. There were significant differences between males and females with regard to fibre – type distribution and relative diameter of the fibres of different type. In females the type I fibres were larger than the type II fibres, in contrast to the normal pattern in most limb muscles in animals and man (Dubowitz & Brooke, 1973). These divergent findings in different species emphasise the difficulty in relating animal data to the situation in human muscles, and indicate the necessity to study human muscles.

1.4.2 Human data.

1.4.2.1 Histochemical Properties.

The first report of the fibre type composition of the human masticatory muscles was by Ringquist in 1971. In this biopsy study a preponderance of type II fibres was seen in the muscles, but no data was presented for the masseter alone. The fibre types of the human masseter have since been classified histochemically using biopsy (Ringquist, 1973*a*,*b*; Serratrice *et al.*, 1976; Ringquist *et al.*, 1982) and autopsy techniques (Serratrice *et al.*, 1976; Vignon *et al.*, 1980; Eriksson & Thornell, 1983). Most of the earlier studies report a greater percentage of type II fibres (range 37 – 79%) than type I in the masseter (Ringquist, 1973*b*; Serratrice *et al.*, 1976; Vignon *et al.*, 1980; Ringquist *et al.*, 1982). As is the case in most human muscles there is a great deal of variation between sites in the same muscle and also between individuals. In the extensive autopsy study by Eriksson & Thornell (1983) the overall proportions of the various fibre types in the masseter of 5 young male subjects were: I, 62.5%; IIA, 2.1%; IIB, 26.7%; IIC, 2.7% and IM, 6.0%. Types IIC and IM are intermediate staining types which are not normally found in human limb and trunk muscles (Dubowitz & Brooke, 1973). It would seem reasonable to conclude that the masseter contains a mixture of type I and II fibres in proportions that may differ markedly among individuals, but on average an approximately even distribution. In view of the greater diameter of the type I compared to the type II fibres in these muscles (see below) it would seem that in general type I fibres comprise the majority of the cross – sectional area in the masseter.

Evidence from a number of the studies mentioned above indicates that the histochemical profile of the masseter (and other masticatory muscles) differs from that normally seen in the limb muscles. The main differences are summarised below:

i) The diameter of the type I fibres is larger than that of the type II fibres in nearly all sites in the muscle (Ringquist, 1973*b*; Serratrice *et al.*, 1976; Vignon *et al.*, 1980; Eriksson & Thornell, 1983). This is in contrast to the normal situation in the limb muscles where the type I and II fibres are either of similar size, or the type II fibres have a larger diameter (Dubowitz & Brooke, 1973).

ii) The diameter of the type II fibres in the masseter (and other masticatory muscles) is generally much smaller than type II fibres in other skeletal muscles (Vignon *et al.*, 1980), and the diameter of type I fibres is also smaller than for

the corresponding type in other skeletal muscles (Ringquist, 1971; Polgar *et al.*, 1973; Eriksson & Thornell, 1983).

iii) The masseter contains a large proportion of fibres with intermediate staining for MATPase (Vignon *et al.*, 1980; Ringquist *et al.*, 1982; Eriksson & Thornell, 1983). Fibres of this type are rare or non-existent in normal adult limb muscles (Dubowitz & Brooke, 1973), but have been seen in large numbers with extreme endurance training of muscles (Jansson & Kaijser, 1977). It was suggested by Eriksson & Thornell (1983) that the existence of this population of fibres may explain the continuous distribution of motor unit contraction times in the human masseter (Yemm, 1977*b*; Goldberg & Derfler, 1977).

iv) It would also appear that the masseter muscle in general does not contain type IIA fibres (Ringquist *et al.* 1982, Eriksson & Thornell, 1983), although these fibres may be found in large proportions in the masseter of some individuals. Type IIA fibres correspond to motor units of the FR type. It has been shown that endurance training tends to increase the proportion of type IIA fibres in a muscle, probably by conversion of the type IIB fibres (Anderson & Henriksson, 1977; Jansson & Kaijser, 1977; Jansson *et al.*, 1978), and it has been suggested that the finding of type IIA fibres in the masseter of some subjects is possibly an adaptation to functional demands (Eriksson & Thornell, 1983).

v) Histochemical methods also reveal that the masticatory muscles do not have the mosaic pattern of fibre type distribution normally found in the limb muscles, but rather have large groups of densely-packed fibres of the same histochemical type (Eriksson & Thornell, 1983; Dubowitz & Brooke, 1973). Such an appearance in skeletal muscle would be considered pathological (Dubowitz & Brooke, 1973). Evidence that this type of appearance does not result from grouping of fibres from the same motor unit has been presented by Stålberg *et al.* (1986).

1.4.2.2 Physiological Properties.

In contrast to the usual situation for other muscles, there is no animal data regarding the physiological properties of individual motor units in the masseter. The only source of this information is from human studies employing the STA technique (Goldberg & Derfler, 1977; Yemm, 1977*b*). In the study by Yemm (1977*b*), the masseter units had a continuous range of contraction times from 24-91 ms, with no evidence for separate populations of fast and slow motor units. There was also no correlation between twitch tension and contractile speed of the

units; in fact in some subjects there was a significant positive correlation, which is the opposite to the pattern reported in human FDI (Milner – Brown *et al.*, 1973*b*) and to the normal pattern seen in the animal muscles studied (reviewed in Burke, 1981*a*). In the study by Goldberg & Derfler (1977) the population of units examined were generally of higher recruitment threshold than the units in Yemm's report. These authors also found a continuous distribution of contractile speeds in the motor unit twitches, but reported that they were distributed over a narrower range (38 – 69 ms), and also found no correlation between twitch tension and contractile speed.

A major limitation of these studies is that fatiguability of the units was not examined. It is therefore impossible to classify the motor units according to their physiological properties with the widely – used system of Burke (1973). This information is essential if the correlations between motor unit physiological and histochemical properties are to be assessed in the human masseter. It is also necessary in order to better understand the functional significance of the unique histochemical profile of the masseter.

Furthermore, there is no information at present regarding fatigue of masseter motor units with either controlled stimulation or voluntary activation in either animal or human studies. Even at the whole muscle level, the available fatigue data for the human masseter is difficult to interpret because of methodological shortcomings (see section 1.5.4.2). This is particularly so for submaximal voluntary contractions. It has been suggested that the fatigue – resistance of the jaw – closing muscles is superior to that of limb muscles during prolonged high – force contractions (van Steenberghe *et al.* 1978; Clark *et al.*, 1984; Clark & Carter, 1985). In the absence of motor unit fatigue data for the masseter, it is unclear whether this indicates an increased fatigue resistance at the motor unit level, or whether other factors (e.g., better oxygenation at high force levels) are responsible.

1.5 Muscle Fatigue

Fatigue is a term which has a number of different usages and interpretations. In the context of the neuromuscular system, fatigue can be defined as the reduction in force generating capacity that occurs during sustained activity (Asmussen, 1979). If one considers the chain of events resulting in the production of force in a muscle, from volition through to the conformational changes in the actin and myosin filaments, it is apparent that there are numerous possible sites for fatigue to occur. These sites can be separated into two categories; a) central sites, or

those involved in the generation and transmission of the nervous impulses to the muscle, and b) peripheral sites, which for the present purposes I will define as lying beyond the neuromuscular junction. It is often useful to distinguish between central and peripheral fatigue, particularly when attempting to quantify fatigue. In the sections below, the possible sites of fatigue are examined in more detail, with particular reference to the likely importance of each site to fatigue of voluntary contractions in humans.

1.5.1 Central Fatigue

1.5.1.1 Maximality of excitation: effort or central excitatory drive.

Early investigators believed that the causes of fatigue were central in origin, and resulted from an inability to fully activate the muscles by the brain, and a progressive diminution of this ability with time (Mosso, 1915; Reid, 1928). The ability to activate a muscle fully may be tested by comparing the force of maximal voluntary contraction with that resulting from supra – maximal stimulation of the motor nerve to the muscle. Using this approach, both Merton (1954) and Bigland & Lippold (1954) were able to demonstrate the ability to activate human adductor pollicis muscle fully during a maximal voluntary contraction for up to 3 minutes. This means that all the motor units in the muscle must be fully activated so that each is producing a fully fused contraction. Belanger & McComas (1981), using a different method, have shown that a number of human muscles can be maximally activated by voluntary effort. The ability to maintain this maximal effort for several minutes proves that central fatigue of effort need not be a limiting factor in fatigue, at least during controlled contractions by highly motivated and trained subjects in laboratory conditions.

1.5.1.2 Maximality of excitation: motoneurone excitability.

Fatigue in the motoneurone could conceivably result in force loss. In animal preparations it is possible to cause motoneurones to discharge at very high rates (100 Hz or more) by direct injection of current into the cell body (Granit *et al.*, 1963). During an injection of a constant current the firing rate of motoneurones rapidly decreases with time, a process termed adaptation by Kernell & Monster (1982*a*). In a prolonged MVC in humans there is a parallel reduction of the surface EMG signal amplitude and force during a maximal contraction which is suggestive of progressive central fatigue, perhaps as a result of motoneuronal adaptation (Bigland – Ritchie *et al.*, 1979; Jones *et al.*, 1979). Indeed the mean firing rate of motoneurones does decrease during an MVC, and the time – course

is similar to that of adaptation of cat motoneurones to constant current injection (Bigland – Ritchie, 1981*b*). Yet in MVC's of up to 3 min duration the loss of force cannot be restored by supramaximal nerve stimulation, which suggests that the reduction in motoneurone firing rate does not result in force loss (Merton, 1954, Bigland – Ritchie, 1981*a*). This is because a decrease in firing rate during an MVC is accompanied by a slowing of muscle contractile speed, resulting in a reduced frequency required for full tetanic tension. It appears that the drop in rate matches the contractile slowing of the muscle so that full tetanus is preserved (Bigland – Ritchie *et al.*, 1983). Recent evidence suggests that this balance between the activation and contractile properties of the muscle is achieved with the aid of feedback from the muscle, rather than being purely a property of the motoneurone (Bigland – Ritchie *et al.*, 1986; Woods *et al.*, 1987).

The reduction in motoneurone firing rate during an MVC does not result in reduced force, but rather helps to preserve force by decreasing the likelihood of muscle activation failure (e.g. failure of neuromuscular transmission) and also preserves control by ensuring that motoneurones are not firing at needlessly supratetanic rates, in which case a large reduction in rate would be necessary before a reduction of force could be accomplished. The balance between motoneurone and muscle mechanical properties means that full activation of the muscle can be achieved without full activation of the motoneurones. This is emphasised by the fact that asynchronous stimulation (similar to voluntary activity patterns) is capable of producing maximal force at much lower rates than is necessary during synchronous activation of all motor units in the muscle (Lind & Petrofsky, 1978; Rack & Westbury, 1969; Edwards, Young *et al.*, 1977). This safety margin significantly reduces the risk of central fatigue in voluntary contractions, since the amount of adaptation to a constant current injection is dependent on the initial firing rate of the motoneurone (Kernell & Monster, 1982*a*). It would seem that central fatigue of motoneurone excitability does not cause loss of force during maximal contractions in the laboratory situation.

1.5.1.3 Neuromuscular Transmission.

Nerves are vastly superior to muscle fibres in their ability to transmit impulses at high frequency for long periods without failure. It would appear that for all practical purposes transmission of impulses along a nerve trunk is inexhaustable. However, conduction block can occur at axonal branch points, where there is a low safety margin for action potential propagation (Smith, 1980; Swadlow *et al.*, 1980). In vivo evidence for this type of failure of propagation has been found in

cat motor units with high rates (80 Hz) of stimulation, but not with stimulation at 10 or 40 Hz, which are comparable to the firing rates of motor units during voluntary activation (Clamann & Robinson, 1985).

The next possible site of failure of transmission of electrical impulses is the neuromuscular junction. Failure of neuromuscular transmission has been demonstrated in rat diaphragm by Krnjevic & Miledi (1958) at frequencies above 50 Hz, but with little likelihood of failure at 10 Hz. This failure was attributed to a diminution of the excitatory post – synaptic potentials and a raised threshold for activation of the muscle fibre, in addition to presynaptic failure in the terminal nerve branches. It has been suggested that neuromuscular block could be significant in fatigue of voluntary contractions (Naess & Storm – Mathisen, 1955), but it is uncertain whether this phenomenon has physiological significance because it has not been demonstrated at stimulus rates comparable to the firing rates of motor units during prolonged voluntary activation. The rapid decline in motor unit firing rates during maximal voluntary contractions also helps to prevent neuromuscular block (see Section 1.5.1.1).

There is conflicting evidence regarding the existence of neuromuscular transmission failure during voluntary contractions in humans, where its existence can be assessed by examining the muscle mass action potential (M wave) evoked by supramaximal shocks to the nerve. Merton used this technique in 1954 and found no decline in amplitude of the evoked M wave during a 3 min maximal contraction of adductor pollicis, and thus concluded that there was no electrical transmission failure. Stephens & Taylor (1972) found evidence for transmission failure using paired stimuli in first dorsal interosseous. The experiments were repeated by Bigland – Ritchie *et al.* (1982) who confirmed Merton's original conclusion. Recently, Bellemare & Garzaniti (1988) have presented evidence for neuromuscular transmission failure during MVC of adductor pollicis using high – frequency stimulus trains, and argued that the failure to detect it in some studies was due to differences in experimental design. It is still not clear whether neuromuscular transmission failure is a limiting factor in fatigue of voluntary contractions, since it has only been demonstrated with synchronous, high – frequency, electrical stimulation; this may not be comparable to asynchronous activation of motor units at lower rates seen in voluntary contractions. Support for the view that failure of neuromuscular transmission does not limit force during a voluntary contraction comes from Merton *et al.* (1981), who found that the force drop during an MVC of adductor pollicis muscle could not be restored by massive

direct stimulation of the muscle fibres themselves, which by-passes the neuromuscular junction.

In summary it appears that central fatigue can be overcome in highly motivated and trained subjects in the laboratory, and need not be responsible for loss of force in this situation. This is not to suggest that central fatigue does not occur. It is likely that progressive central fatigue of effort is an important determinant of fatigue during everyday activities, sports and some diseases. The important point about the ability to exclude central causes of fatigue during a fatigue test is the demonstration that there are fatigue processes occurring in the muscles themselves. These peripheral factors are discussed below.

1.5.2 Peripheral Fatigue.

The sites of peripheral fatigue lie within the muscle. The processes which may be affected during activity can be divided into those concerned with a) muscle fibre excitability, and b) excitation contraction coupling.

1.5.2.1 Muscle Fibre excitability.

Following the activation of the muscle fibre at the neuromuscular junction an action potential is propagated along the muscle fibre, and via the T-tubule system the excitation is transmitted rapidly into the interior of the muscle. Changes in fibre excitability may result in loss of force if they are sufficient to prevent the spread of the excitation along all or part of the fibre, with the resultant failure to activate all the contractile elements. An alternative hypothesis is that an altered action potential may not be able to activate the contractile elements maximally.

During high-frequency stimulation of muscle there are changes in muscle fibre excitability which result in progressive changes in the propagated action potential, and which, if continued at high frequencies for long enough, may result in failure of action potential propagation (Krnjevic & Miledi, 1958). Changes in action potential waveform are also seen during forceful voluntary contractions. The main changes are a progressive reduction in amplitude and increase in duration of the muscle action potential (Bigland-Ritchie *et al.*, 1979; Jones, 1981; Milner-Brown & Miller, 1986). The change in duration of the potential is indicative of a change in conduction velocity of the muscle fibres (Lindström *et al.*, 1970). The rapid recovery of the action potential following cessation of stimulation suggests that these changes in muscle membrane excitability are due to changes in the sodium/potassium balance in the extracellular fluid rather than processes involved

in energy supply (Krnjevic & Miledi, 1958; Bigland – Ritchie *et al.*, 1979; Jones, 1981).

What is the relevance of these electrical changes to fatigue of voluntary contractions in humans? Total blocking of fibre action potentials has been observed during high – frequency stimulation of muscle in vitro (Lüttgau, 1965) and in vivo (Bigland – Ritchie *et al.*, 1979), and is logically associated directly with force loss. However, there is no evidence that blocking of fibre potentials occurs during voluntary activation of muscles. Although changes in fibre action potentials have been found to accompany fatigue during voluntary contractions, a causative relationship between changes in amplitude or duration of fibre action potentials and force loss has not been demonstrated. In fact, it has been stated that providing an action potential is capable of propagation along the muscle fibre, it is capable of fully activating the contractile apparatus (Falk, 1961).

In voluntary contractions, the relationship between electrical and mechanical events during fatigue has been studied at the whole muscle level. Unfortunately, in most cases the experimental design has not allowed direct comparison of force changes with electrical changes under verifiable conditions of constant excitation. This is essential in order to objectively measure fatigue (see section 1.5.3). The relationship between electrical and mechanical events during fatigue has not been studied at the motor unit level in voluntary contractions. This approach has the advantage that fatigue can be objectively measured under conditions of constant excitation (constant firing rate), and hence direct comparison made with concurrently – occurring electrical events in the muscle. This helps to establish the causes of force loss, and also indicates how well electrical events may reflect contractile fatigue (see Chapter 6).

1.5.2.2 Excitation – contraction coupling:

For the purposes of this discussion, excitation – contraction coupling will be defined as the processes involved in the production of force following the electrical activation of the muscle fibre. At the present time, these complex processes are not well understood. Briefly, depolarisation of the fibre surface membrane leads to a rapidly – conducted depolarisation into the interior of the cell, aided by the T – tubule system. It is believed that depolarisation of the T – tubule membrane acts to cause the release of calcium ions (Ca^{++}) from the terminal cisternae of the sarcoplasmic reticulum into the internal environment of the muscle cell. This extremely high intracellular concentration of Ca^{++} triggers binding of the Ca^{++} by the troponin molecules on the actin filament. This

causes a configurational change in the tropomyosin, and exposes the attachment site of the myosin cross – bridge on the actin filament. The actin – myosin cross – bridges undergo a conformational change so that the actin and myosin filaments slide past one another, and force is produced. After each sliding movement the actin – myosin bond is broken and another cycle begins. Each cycle requires the dephosphorylation of one molecule of adenosine tri – phosphate (ATP). It has been shown in frog muscle that the rate of splitting of ATP is proportional to the force produced (Dawson *et al.*, 1978). In order for the muscle to relax, Ca^{++} must be removed from the interior of the cell. This process also requires ATP as it is accomplished by an active transport Ca^{++} pump from the interior of the cell back into the sarcoplasmic reticulum.

Disruption of the processes involved in excitation – contraction coupling, and subsequent fatigue, could be the result of several inter – related consequences of contraction. The first is a reduction in the rate of supply of ATP, which is needed for both membrane and contractile functions. This may come about due to a lack of, or reduced, substrate for its production, or from accumulated products of contraction (e.g. H^+) which may inhibit further ATP production by inhibiting the enzymes involved in glycogen breakdown by the anaerobic pathway. Accumulation of H^+ ions may reduce the Ca^{++} – activated actomyosin reaction by changing the shape of the tropomyosin complex, and also reduce myosin – ATPase activity so that the rate of crossbridge cycling is reduced. It has also been speculated that pH is important in determining fibre membrane excitability and action potential propagation. These latter effects of lowered pH result in a decreased utilisation of ATP, and it has been suggested that intracellular pH may perform a regulatory role during muscle fatigue by acting as one of the fail – safe mechanisms that ensures that contractile failure occurs before the level of ATP in the muscle drops to a dangerous level (Edwards, 1981). An increase in extracellular K^+ concentration (or depletion of Na^+) is also considered to have important effects on membrane excitability (Krnjevic & Miledi, 1958; Jones, 1981), and may also act as a protective mechanism by limiting action potential propagation before ATP is depleted.

It is possible to observe impaired excitation – contraction coupling which does not appear to be directly related to muscle energy supply or the accumulation of metabolic by – products. This is "low – frequency fatigue" which is characterised by a selective loss of force at low frequencies after continuous activation, while the force of maximal contraction is unimpaired (Eberstein & Sandow, 1963; Edwards, Hill, *et al.*, 1977; Kugelberg & Lindegren, 1979). The defect here is not in the

force – producing capacity of the muscle contractile elements themselves as the force decrement can be overcome with maximal activation. This fact and the very slow time course of recovery (of the order of hours) leads to the conclusion that the defect is not due to impaired rate of ATP supply or the accumulation of metabolic by – products. In most cases the membrane excitation appears normal. The problem is a reduced force output for each individual action potential. It has been speculated that the defect may be a reduced release of Ca^{++} ions with each membrane depolarisation, or possibly impaired transmission in the T – tubule system as a result of structural damage (Edwards, 1981). Although apparently not directly related to metabolic factors, it is significant that the resistance to the development of this type of fatigue in rat fast and slow motor units was correlated with the levels of a marker of oxidative enzyme activity (succinate dehydrogenase) in the muscle fibres of the units (Kugelberg & Lindegren, 1979). It would appear that a low capacity for oxidative metabolism is an important factor in the development of low – frequency fatigue.

In summary, the causes of excitation – contraction coupling deficits leading to fatigue are not well understood. It is thought that these processes are of particular importance during fatigue of normal everyday contractions, although it is difficult to assess this accurately. Part of this difficulty in voluntary contractions is related to the difficulties encountered in fatigue testing in this situation. This issue is addressed in the following Section.

1.5.3 Fatigue Testing

The accurate measurement of fatigue is obviously important in order to attempt to evaluate it's underlying causes. The term "fatigue" has a wide range of usages and interpretations in the English language. In many human studies, accurate measurement of fatigue has been hampered by a failure to design an experiment consistent with a meaningful definition of fatigue. The previously quoted definition of fatigue by Asmussen (1979), which is a reduction in force producing capacity of the Neuromuscular system during sustained activity, is adequate as a description of the process, but needs to be clarified to serve as a basis for fatigue measurement.

In the preceding Sections, the possible sites for fatigue have been mentioned. These can be broadly categorised as central or peripheral sites. It is often useful to distinguish between central and peripheral fatigue, particularly when attempting to quantify fatigue. Central fatigue is characterized by a reduction of electrical activation of the muscle, accompanied by (but not necessarily causing) force loss.

Peripheral fatigue is characterized by a reduction in force output with constant excitation of the muscle. When measuring fatigue it is desirable to assess, and if possible control the level of excitation of the muscle in order to differentiate central from peripheral causes of force loss. For most practical purposes, it is desirable to test fatigue under conditions of constant excitation (i.e. in the absence of central fatigue), so that fatigue may be objectively measured in terms of loss of force. Under conditions of constant excitation, the causes of fatigue lie within the periphery, and may be termed contractile fatigue. Therefore, a more precise definition of peripheral or contractile fatigue, and one to be preferred in fatigue testing, is "*a reduction of force output of the muscle while it receives a constant level of excitation*". The following review is limited to fatigue of isometric contractions.

1.5.3.1 Fatigue Testing in animals.

In animal experiments it is a relatively simple matter to measure fatigue under conditions of constant excitation by electrically stimulating either the whole muscle, or single motor units, and recording the force output. This has resulted in a straightforward approach to fatigue testing which is consistent with the above ideals.

The usual approach is to dissect and stimulate individual motor nerve axons, so that the mechanical responses of single motor units are studied. However, even with this approach care must be taken that the stimulation paradigm ensures constant activation of the muscle. Continuous high – frequency stimulation of motor units at rates of 80 Hz or more results in rapid loss of force due to disruption of muscle activation (Burke, 1981*a*; Clamann & Robinson, 1985; Gardiner & Olha, 1987; Sandercock *et al.*, 1985). This failure of activation can be assessed by observing changes in the muscle fibre action potential reflected in the EMG signal. The significance of this type of fatigue to the situation in voluntary contractions is dubious, as motor units do not fire continuously at rates much above 30 Hz during voluntary activation (De Luca, 1979).

Consequently, contractile fatigue is more usefully assessed in motor units by stimulation at lower rates. The test introduced by Burke (1967), or modifications thereof, is the most commonly used. In this test units are stimulated with trains of 13 pulses each at 40 Hz, repeated once per second, for 2 or more minutes. This is generally supposed to result in failure of the contractile apparatus, with little activation failure. However, there are a number of reports showing EMG changes suggestive of activation impairment in motor units when this test is used

(Reinking *et al.*, 1975; Goslow *et al.*, 1977; McDonagh *et al.*, 1980). Nevertheless, the test has been valuable and widely used to measure and compare fatigue in motor units of a number of different muscles (reviewed in Burke, 1981*a*).

Stimulation at even lower rates has been used by others to measure contractile fatigue without any signs of activation failure (Edström & Kugelberg, 1968; Kugelberg & Edström, 1968; Sandercock *et al.*, 1985). With continuous activation at rates below 20 Hz, contractile fatigue can usually be demonstrated without any change in the EMG signal (Kugelberg & Lindegren, 1979).

The many motor unit studies in animals using a constant excitation approach have led to a greater understanding of the factors influencing fatiguability, for example the biochemical correlates within the muscle fibres (see Sect. 1.2). This has been possible because the tests used have provided a standardised, objective measure of fatigue which facilitates comparison between individuals, muscles and species. However, any paradigm involving electrical stimulation is artificial, and it may also be argued, unphysiological. Data obtained from animals may not be directly applicable to humans. For these reasons it is necessary to also test fatigue in humans, preferably during voluntary activation of the muscles.

1.5.3.2 Fatigue Testing of human voluntary contractions.

Unfortunately, fatigue testing of human voluntary contractions is not as straightforward as fatigue testing in animals with electrical stimulation. The ideal constant excitation approach, in which fatigue can be simply described in terms of loss of force, has not been widely used in humans. Of the whole muscle studies, probably only the study of fatigue during an MVC has employed this approach. The maximal force a subject can exert is a notoriously variable quantity, but constant (maximal) excitation can be verified by intermittent supra – maximal nerve shocks (e.g. Bigland – Ritchie, 1981*a*). Using this approach, central fatigue can be overcome by highly motivated, trained subjects in some (but not all) muscles. Therefore, under these conditions, the decrease in force represents contractile fatigue. The MVC test is valuable to quantify this fatigue since it provides an index of contractile fatigue that is reproducible, and also allows comparison between subjects or muscles at identical levels of relative activation. The deficiencies of the MVC paradigm are that it is so stressful to the subject that it is not of practical use clinically, and that it can only give information about fatigue occurring at one level of excitatory drive (i.e. maximal). It gives no information about the fatigue processes occurring in low – force contractions (e.g. "low – frequency fatigue", Sect. 1.5.2.2).

The measurement of fatigue in submaximal voluntary contractions in humans has been hampered by the difficulty in assessing the level of excitation of the muscle, with the result that the relationship between the level of excitation and force (on which objective fatigue measurement is based) has usually not been determined unambiguously. In one paradigm, the subject keeps the force constant, and an increase in amplitude of the surface EMG signal (measured as either: integrated rectified EMG, smoothed rectified EMG, or the RMS amplitude) is interpreted as indicating an increase in excitation of the muscle to compensate for fatigue (e.g., Edwards & Lippold, 1956; Maton, 1981). This interpretation relies critically on the assumption that the contribution of individual motor units to the surface EMG is invariant with time. Several recent reviews point out that in forceful, submaximal contractions, the contribution of motor units to the surface EMG signal does change because of a change in duration of their action potentials (Bigland – Ritchie, 1981*b*; De Luca, 1984). Furthermore, one cannot be certain that the same units are firing at the same rate throughout a contraction in which the total surface EMG amplitude remains unchanged. The complex nature of the surface EMG signal means that measures of its amplitude do not give a precise measure of the level of excitation of the muscle.

The inability to reliably control the level of excitation of the muscle in a submaximal contraction has led to more indirect methods of fatigue testing in this situation. To be meaningful, any measure of contractile fatigue must relate force to the level of excitation of the muscle, yet many of these other fatigue tests do not do this. For example, the endurance time is not an objective measure of fatigue (see Sect. 1.5.4.2). Another common approach is to infer fatigue from changes in the power spectrum of the surface EMG signal. A shift in the power spectrum towards low frequencies has been shown to accompany fatigue during forceful contractions (Kadefors *et al.*, 1968; Lindström *et al.*, 1970). Most investigators consider that the major component of the frequency shift in the SEMG results from the increase in duration of the motor unit action potentials due to a slowing of fibre conduction velocity (Lindström *et al.*, 1970; Mills, 1982; Kranz *et al.*, 1983; De Luca, 1984; Eberstein & Beattie, 1985). In maximal contractions, where fatigue can be directly measured from the loss of force (constant excitation paradigm), there appears to be a good correlation between the shift to low frequencies and contractile fatigue (Mills, 1982). However, the nature of the relationship between the two is not clear, as during recovery the maximal force recovers quicker than the frequency shift in the SEMG signal (Mills, 1982),

and the force from low-frequency stimulation may remain impaired for some time after the SEMG signal has returned to normal (Moxham *et al.*, 1982).

The shift in the power spectrum of the surface EMG signal towards low frequencies has also been used to infer contractile fatigue during sub-maximal contractions (Lindström *et al.*, 1977; Häkkinen & Komi 1983), including Lindström & Hellsing (1983) and Naeije (1984) in the masseter muscle. The complex nature of the relationship between the EMG signal and force has led to some ambiguity when power spectrum changes are used to assess fatigue in submaximal contractions. The use of a constant-force paradigm in this type of experiment makes direct measurement of fatigue in terms of loss of force impossible. In some studies force has not even been measured, let alone controlled. The assumption is made that since the changes seen in the power spectrum of the surface EMG during moderately forceful sub-maximal contractions are similar to those occurring during maximal contractions, then contractile fatigue has occurred. This is probably true, but it is debatable whether contractile fatigue may be quantified from the EMG power spectrum alone, particularly in submaximal contractions, as has been suggested (Lindström *et al.*, 1977; Lindström & Hellsing, 1983). A direct cause and effect relationship has not been demonstrated between the loss of force and changes in the power spectrum of the surface EMG, and the dissociation between EMG power spectrum changes and force under certain conditions (e.g. Mills, 1982; Moxham *et al.*, 1983) make this approach questionable.

The ideal of testing whole-muscle fatigue under conditions of constant excitation is difficult to achieve in a voluntary contraction. Yet, it is easily achieved for a single motor unit under voluntary control, and the force output of the unit can be determined with STA. This approach is potentially much more informative than whole-muscle studies, but has not been widely used. There is only one previous study of fatigue of single units during a voluntary contraction in humans (Stephens & Usherwood, 1977; see Section 1.3.2). This approach was chosen for the present study because it was considered to allow ready comparison of objective measures of fatigue between muscles, and also because fatigue data for single units in the masseter was not available. It had the added advantage that fatigue was measured concurrently with changes in the (single unit) EMG under conditions of constant excitation, which facilitated study of the relationship between force and the EMG signal during fatigue.

1.5.4 Fatigue in the masseter muscle.

1.5.4.1. Animal Data

There is very little data on fatigue of the masticatory muscles in experimental animals. To the best of my knowledge, the only study where a fatigue test using controlled stimulation has been applied to the masseter in animals is that of Taylor *et al.* (1973), in which isolated strips of cat masseter and temporalis muscle were stimulated with pulse trains of 55 – 90 Hz, for 330 ms, repeated once per second. Although detailed results were not presented, it was reported that the typical finding was a rapid reduction in force output during the first minute to about 25% of the initial level, and then a gradual decline over the next 2 – 3 minutes. These results were deemed to be consistent with the relative proportions of fibre types determined using histochemical techniques in the same study (predominantly type II fibres). More detailed data, in particular motor unit fatiguability, which has been widely studied in other animal muscles, is not available for the masticatory muscles. This is presumably related to the difficulty in gaining access to the motor nerves in these muscles without damaging the muscle itself. However, even if it were available, it is probable that the animal data would not be particularly enlightening on the properties of human masseter because there appear to be significant differences in the fibre type composition of the human and animal muscle (human masseter is predominantly type I whereas in the cat and rat it is predominantly type II; also human masseter appears to lack the type IIA fibres (FR units)). The masseter in the rhesus monkey does appear to have a similar histochemical profile to humans (Maxwell et al., 1979) and might therefore yield valuable physiological data, but this has not been attempted.

1.5.4.2 Human Data.

A number of reports have appeared in the literature concerning "fatigue" of the jaw muscles (van Steenberghe *et al.*, 1978; Christensen, 1979; Christensen & Mohamed, 1983; Christensen *et al.*, 1985; Palla & Ash, 1981; Naeije & Zorn, 1981; van Boxtel *et al.*, 1983; Naeije, 1984; Clark *et al.*, 1984; Clark & Carter, 1985; Kroon *et al.*, 1986). Many of these studies are of limited value because of a failure to relate force changes to muscle excitation, and hence do not give objective measures of fatigue. The approaches to fatigue testing in the human masseter can be grouped into three broad categories, which will be considered below. Of these approaches, only the measurement of the force of maximal contraction as an indicator of fatigue approaches the above ideals (see iii) below).

i) The endurance time, or the time elapsed until the subject is unwilling to continue a required task, has been used as an index of fatigue in both maximal (Christensen, 1979; Christensen & Mohamed, 1983; Christensen *et al.*, 1985; Palla & Ash, 1981) and submaximal biting (Naeije, 1984; Clark & Carter, 1985). Endurance has been confused with resistance to fatigue in most of these studies, and although they may be related, they are not the same. Endurance is defined in the Oxford Dictionary as the ability to withstand prolonged strain, and clearly is a highly subjective measure. During sustained biting at force levels varying from 25 – 100% of maximal it was demonstrated by Clark & Carter (1985) that the subject's endurance was limited by pain. However, the ability to perform intermittent maximal contractions at intervals during sustained submaximal biting was unaffected, indicating that fatigue or loss of power was not the limiting factor. Pain in contracting muscle is believed to be related to an increased concentration of the metabolic by – products of contraction such as lactic acid and potassium (Mense, 1977; Mills & Edwards, 1984), as a consequence of contraction – induced occlusion of blood flow through the muscle. The build up of these products may be influenced by the fatigue resistance of the muscle, but the limiting factor in endurance is subject motivation. In fact, in those studies where force was not controlled (e.g., Christensen, 1979; Christensen & Mohamed, 1983; Christensen *et al.*, 1985; Naeije, 1984; Palla & Ash, 1981) it could be argued that a long endurance time may indicate a *poor* fatigue resistance. Since muscle pain is the limiting factor to endurance, and pain results from force – related occlusion of blood flow, it is conceivable that an individual whose muscle fatigues so rapidly that the intramuscular pressure drops below the level where blood flow is occluded would be spared from pain and thus able to continue the contraction for a longer period. It is obvious that endurance time is not an objective measure of muscle fatigue.

ii) The shift in the power spectrum in the surface EMG towards greater low – frequency energy content with muscle fatigue (Lindström *et al.*, 1970; Mills, 1982) has been used in attempts to quantify masseter fatigue in humans (Palla & Ash, 1981; Naeije & Zorn, 1981; Lindström & Hellsing, 1983; van Boxtel *et al.*, 1983; Naeije, 1984; Kroon *et al.*, 1986). All of these studies show the familiar relative increase in low frequency content in the EMG signal during a variety of fatiguing tasks, yet to be meaningful, any index of fatigue must consider force, and studies in which force has not been measured (Palla & Ash, 1981; Naeije & Zorn, 1981; van Boxtel *et al.*, 1983; Naeije, 1984; Kroon *et al.*, 1986) are of little value in quantifying fatigue. The change in the power spectrum of the surface EMG

signal is not sufficient to quantify fatigue in terms of a loss of force – producing capacity (see Section 1.5.3.2).

iii) The ability to produce maximal force during repeated contractions appears to give an reasonably objective measure of fatigue. Although not ideal because of reproducibility problems associated with any maximal force measurement, with due care this approach at least allows some quantitative comparisons to be made between subjects and different muscles. In a comparison of the ability to maintain repeated maximal contractions of short duration, van Steenberghe *et al.* (1978) found that the jaw – closing muscles were not affected under conditions in which the muscles producing hand – grip, and arm – flexion forces were significantly weakened. The ability to preserve maximal force – producing capacity during and following prolonged submaximal contractions at various proportions of maximal force also appears to be greater in the jaw – closing muscles than other muscles studied (Clark *et al.*, 1984; Clark & Carter, 1985).

In summary, there is not a great deal of reliable information on the fatiguability of the human masseter. From maximal biting force (MBF) experiments, there is evidence that the masseter is more resistant to fatigue of maximal contractions than non – masticatory muscles (van Steenberghe *et al.*, 1978; Clark *et al.*, 1984; Clark & Carter, 1985). Much of the available data for fatigue of submaximal contractions in masseter is not quantitative. There is no data at all on the fatigue properties of masseter single motor units in either animals or humans, which makes it difficult to speculate on the reasons for the apparently superior fatigue resistance of the masseter in certain tasks.

1.6 Control of motor unit activity.

The production of force by a muscle is achieved by activation of the functional quanta of muscle; the motor units. Control over the level of force produced during a contraction is achieved by variation of the number of active motor units (recruitment or de – recruitment), and/or by variation of the firing rate of the active units. The concept of recruitment was first described by Liddell & Sherrington (1925), and the importance of firing rate modulation in control of muscle force was emphasised shortly afterward by Adrian & Bronk (1929).

1.6.1 Orderly Recruitment and the Size Principle.

An important question is: How does the central nervous system select the number of active units, and their firing rates, in a combination which is appropriate for

the task at hand? An early concept was that the active units may be rotated during prolonged contractions, perhaps as a means of minimising fatigue (Forbes, 1922). However, this idea soon fell into disfavour once intramuscular recording of motor unit activity became commonplace (Adrian & Bronk, 1929; Denny – Brown, 1929; Smith, 1934). The suggestion implicit in the concept of rotation of motor unit activity is that units may be individually controlled by the CNS. That is, the desired force may be achieved by any combination of the motor units at the disposal of the CNS. However, it has been recently pointed out that such an arrangement, whereby units are recruited in random order for a particular task, would be needlessly complicated and very time – consuming in terms of central processing; in fact the processing requirements for even a single muscle such as soleus exceeds the total number of cells in the brain (Henneman & Mendell, 1981).

The orderly nature of motor unit recruitment was recognized by Denny – Brown & Pennybacker (1938), who observed a consistent and reproducible recruitment order for motor units in voluntary contractions, and furthermore observed that the units recruited early during a progressive, slow increase in force were invariably smaller than those recruited later in the contraction. Extensive experimental evidence for a relatively fixed recruitment and de – recruitment order based on the size of the motor unit has been provided by Henneman and colleagues from the late 1950's until the present day, and has come to be termed the "size principle" of motor unit recruitment (reviewed in Henneman & Mendell, 1981). Evidence for a size – structured motor unit recruitment hierarchy came from studies with the decerebrate cat in Henneman's laboratory (Henneman *et al.*, 1965*a*,*b*), in which it was demonstrated that size – related motor unit recruitment was very reproducible and independent of the source of input. In a later study with the decerebrate cat it was shown that motor units exhibited a critical firing level, so that all units responded reliably to input drive above their critical level, and failed to respond to input drive below their critical level, or functional threshold (Henneman *et al.*, 1974). In Henneman's Law of Combination, at any level of net excitatory drive, the last – recruited unit is the largest active unit, and all smaller units continue to discharge as the drive is increased (Henneman *et al.*, 1974). The essence of the scheme of motor control advanced by Henneman is that motoneurones within a muscle pool receive qualitatively identical inputs, with the response of each motoneurone determined by its biophysical properties (cell size being the major determinant in Henneman's view). Such an arrangement constrains the possible combinations of units to perform a task into a consistent subset based on the

response of the motoneurones to a non-specific excitatory drive to all motoneurones in the pool, and greatly simplifies the amount of central processing needed for motor control. Subsequent study of the recruitment and firing patterns of motor units in human voluntary contractions have supported the general principles of this control scheme, albeit with some qualifications.

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

Whatever the critical determinants of motoneurone functional threshold prove to be, it is apparent that motoneurone size is a major factor. It has been shown by Henneman and others that the processes resulting in recruitment and rate modulation of motor units during a voluntary contraction are inexorably linked, so that a single strategy combining both facilities is used to control force output in all muscles. The result is a system of motor control that is reproducible (and therefore predictable) and yet frees the brain from the onerous task of individual control of the motor units.

1.6.2 Interaction between Recruitment and Rate Modulation.

The activity of units in the muscle is linked so that a change in force is accomplished by a command signal that acts non-selectively on the units in the pool. Several reports have demonstrated that the firing rates of active units increase proportionally as force is increased (Person & Kudina, 1972; Milner-Brown *et al.*, 1973*c*; Tanji & Kato, 1973*a*,*b*; Monster & Chan, 1977). De Luca *et al.* (1982*b*) have shown that the firing rates of active motor units in a muscle are highly correlated, even during an attempted constant-force contraction, which led them to propose that all motor units in the muscle are subject to "common drive".

The source of this common drive must come from synaptic inputs which are widely-distributed to all the motoneurones in the pool; an arrangement that is central to Henneman's size principle and law of combination, and for which there is considerable circumstantial evidence, at least for excitatory connexions (reviewed by Miles, 1987).

The relative contribution of motor unit recruitment and rate modulation to the control of force varies between muscles. As a general rule, recruitment is complete in small muscles of the hand below about 50% of maximal force (Milner-Brown *et al.*, 1973*b*; De Luca *et al.*, 1982*a*; Kukulka & Clamann, 1981). Further increase of force in these muscles is accomplished by increasing the firing rate of the active units. Larger muscles such as biceps, deltoid and brachialis which are used for powerful contractions rely on recruitment over a greater proportion of the force range, perhaps as high as 80% (Kanosue *et al.*, 1979; De Luca *et al.*, 1982*a*; Kukulka & Clamann, 1981). The corollary of this is the observation that the dynamic range of steady firing rates is much greater in motor units of rate-coded muscles such as first dorsal interosseous (9-40 Hz; De Luca *et al.*, 1982*a*), than in larger muscles relying primarily on recruitment such as soleus (6-10 Hz; Mori, 1973). It has been suggested that rate-coding offers advantages when fine control of force is required, such as in the finger muscles, because it allows smaller increments to be added to the total force. The human masseter is apparently an exception to this general rule. Although it is a powerful muscle (the combined jaw-closers can produce over 4400 N of force; Gibbs *et al.*, 1986), it is heavily reliant on rate-coding (mean motor unit firing rates vary from 6-26 Hz; Derfler & Goldberg, 1977).

1.6.3 Evidence for modification of the Recruitment Order.

Since the demonstration of the size principle of recruitment, a great deal of effort has been devoted to finding exceptions to the rule. Despite earlier reports that subjects could be trained to exert individual control over motor units (Harrison & Mortenson, 1962; Basmajian, 1963), it is now recognized that truly independent voluntary control over single motor units is not possible (Henneman *et al.*, 1976).

Reversal of recruitment order for units of close functional threshold are not considered to be significant, but merely a consequence of the "noise" in the system. Two general conditions appear capable of producing a significant alteration of the normal recruitment order. The first is modulation of cutaneous afferent input, which has been noted to differentially affect large and small units in both animals (Kanda *et al.*, 1977; Kernell & Sjöholm, 1975) and humans

(Grimby & Hannerz, 1968 & 1976; Stephens *et al.*, 1978; Garnett & Stephens, 1981; Datta & Stephens, 1981). The interpretation of these findings is that cutaneous afferent synaptic inputs are apparently not distributed amongst the motoneurones in the pool in the same manner as central excitatory or spindle Ia inputs.

The second is in muscles which perform more than one function. Examples of recruitment reversals during different tasks have been reported in abductor pollicis brevis (Thomas *et al.*, 1978), first dorsal interosseous (Desmedt & Godaux, 1981) and rectus femoris (Person, 1974). It is not known at present whether these recruitment reversals arise from a re-ordering of the central command signal, or whether the changes arise from alteration of afferent inputs to the motoneurones in the different tasks.

Although the recruitment order is apparently flexible for different tasks, it is not clear from the available data whether the capacity to alter recruitment order is utilized in conscious humans during the performance of a particular task. The evidence for recruitment de-ordering has mainly come from electrical stimulation, which is not a physiological stimulus, or in reduced animal preparations. Investigation of this question in humans is difficult. There are problems with reliable identification of action potentials from the same motor unit during prolonged contractions, or under varied experimental conditions (e.g., changing muscle length) because of the sensitivity of the intramuscular electrodes to changes in fibre/electrode geometry. Apparent inactivity of a motor unit may be due to changes in recruitment threshold, or due to a change in electrode position, so that the unit's electrical activity is no longer detected. There is the added problem of adequately controlling the task, as changes in limb orientation can affect recruitment order. Reliable measurement of recruitment shifts are difficult because the recruitment threshold is commonly defined in terms of force output (as a relative measure of excitatory drive), yet the force measured in human studies is invariably torque around a joint. Very few muscles act alone on a joint, and torque around a joint may not give an accurate measure of the force output (excitatory drive) of the particular muscle containing the motor units under study, as the force output of other muscles acting around the joint (both synergists and antagonists) contribute to the net torque, and the relative contribution of each may change. In prolonged contractions, contractile fatigue also becomes a factor. For these reasons, comparison of recruitment thresholds from trial to trial must be interpreted with great care. Comparison of recruitment rank order can be made on a relative basis within trials, but an indication of absolute force

thresholds are usually sought to give an indication whether major shifts in recruitment order have occurred.

Since there is a link between the factors determining whether a motoneurone reaches firing threshold or not, and the rate at which it discharges at a particular level of excitatory drive, in short – term contractions one can infer the recruitment order of concurrently – active motor units from their relative firing rates (Tanji & Kato, 1973*b*; Derfler & Goldberg, 1977). It is not known whether these size – structured firing – rate relationships are preserved during continuous voluntary activation of muscle, or whether they may be modified, in an analogous manner to the alteration of recruitment thresholds. Rather than use force as a control of excitatory drive (for the reasons mentioned above), a better way to investigate this question is to control the firing rate of one unit, and observe the firing patterns of other concurrently – active units during the contraction. This approach was used in the present study to assess the stability of motor unit activation patterns during prolonged voluntary activation in the masseter (see Chapter 9).

1.7 Synchronization of motor unit discharge.

Synchronization may be defined as the tendency of a motor unit to discharge at or near the time of discharge of other motor units. An assessment of the degree of synchronization is particularly important when use of the STA technique is contemplated, as in the present series of investigations, because the accuracy of this technique is dependent on the relative statistical independence of the firing events in the trigger unit, compared to other active units in the muscle.

Early studies described changes in the gross EMG signal which were interpreted as resulting from synchronous discharge of motor units. Lippold *et al.* (1957) found a grouping of activity of motor unit action potentials at a firing rate of about 9 per second in a number of human muscles. This grouping was more pronounced as the muscle fatigued. This was interpreted as evidence that motor units were tending to fire synchronously, and that this tendency increased with time. Others have claimed to observe synchronization by noting the occurrence of large periodic oscillations in the EMG signal as the contraction continued (Missiouro *et al.*, 1962; Mori, 1973). The validity of inferring synchronization of motor unit activity from these changes in the EMG signal was questioned by Taylor (1962), who argued that the grouping of motor unit activity was a chance phenomenon which arose because the units were firing at similar rates.

More objective attempts at quantifying synchronization utilise some form of cross – correlation procedure (Moore *et al.*, 1966). Several investigators have reported evidence of synchrony by cross – correlating the gross EMG signal from different sites within the same (Person & Kudina, 1968) and different muscles (Person & Kudina, 1968; Loeb *et al.*, 1987). It is still questionable whether this approach actually detects synchronization, or whether it reflects other correlated properties of groups of motor units, such as common modulation of firing rate (De Luca *et al.*, 1982*b*; De Luca & Mambrito, 1987), and the tendency of motor units to discharge at similar mean rates. In any event, cross – correlation of EMG signals has other severe limitations which limit its usefulness as a measure of the degree of synchronization of motor units. These are related to the fact that the discharge properties of groups of units must be inferred from the complex interference pattern of the EMG signal. Specific problems include the non – linear summation of different units to the overall signal, and time – dependent changes in the contribution of individual units. These deficiencies confound attempts to infer changes in the degree of synchrony with time, or at different levels of excitatory drive. In addition, there is at present no statistical measure of the degree of significance of any observed correlation in the EMG signals.

Another method of assessing motor unit synchronization employs STA of the rectified and unrectified surface EMG, and has been used to assess synchronization in the human FDI (Milner – Brown *et al*, 1973*a*; Milner – Brown *et al.*, 1975) and also human masseter (Yemm, 1977*b*). With this technique most subjects show little evidence of synchronous motor unit activity, although in some subjects broad synchronization was revealed in FDI. The advantage of this technique over the previously mentioned EMG methods is that the cross – correlation is referenced to the activity of only one motor unit, and is thus interpreted with greater ease and reliability. It does give an estimate of the overall tendency for correlated discharge of other units in the muscle with respect to the reference unit. However, the technique suffers from the same limitations as all methods reliant on the contribution of motor units to the complex EMG signal; it is not strictly quantitative since the contribution of each unit to the EMG signal is not equal, and may change with time quite independently of a change in the degree of motor unit synchronization. The method is also unsuitable for assessing synchrony that is very closely time – locked to the triggering unit, such as the short – term synchronization of ± 3 ms which has been found in some muscles (see below).

The most direct and unequivocal methods for detecting motor unit synchronization are those that consider the times of firing of individual motor units with respect to each other. Buchtal & Madsen (1950) were the first to apply a form of cross – correlation analysis to motor unit spike trains and found that in most muscles examined in normal subjects the incidence of motor unit pairs showing more coincident firings than predicted by chance was less than 20%. This was higher in some small hand muscles and also in some patients with neurological diseases. Taylor (1962) performed a similar analysis to assess synchronization in a number of different human and cat muscles, and did not find evidence of significant synchronization in any of the muscles studied.

A refinement of these earlier methods is the cross – correlation interval histogram (Moore *et al.*, 1966). In this procedure a histogram is produced of the time of firing of other concurrently – active units with respect to the firing of a reference unit. In the absence of any tendency toward synchronization of the two units the cross – correlation histogram will be flat (Moore *et al*, 1966). If a tendency toward synchronization exists, there will be a peak in the cross – correlation histogram around the time of firing of the reference unit. The histogram can be used to detect synaptic connections between motoneurones (Moore *et al.*, 1970).

Using cross – correlation of firing times, Sears & Stagg (1976) found evidence of short – term synchronization ($\pm$3 ms) in groups of intercostal motoneurones in the cat responding to voluntary synaptic drive. This type of synchrony is very weak, requiring a large number of counts to be visible. It was argued that this type of synchronization is an expected consequence of motoneurones sharing a large number of excitatory synaptic connections, an hypothesis which was confirmed for intercostal motoneurones by a series of experiments in which small depolarisations in the synaptic noise of other motoneurones were detected by averaging with respect to the discharge of a single motoneurone (Kirkwood & Sears, 1978).

In humans, several studies report no evidence for synchronization of motor unit activity in a number of muscles using cross – correlation of motor unit spike trains (Kranz & Baumgartner, 1974; Goldberg & Derfler, 1977). However, other studies have found evidence of synchronization in the same, and other muscles with this technique. Dietz *et al.* (1976) found narrow peaks in the cross – correlation histograms in 70 of 87 pairs of units from human FDI, gastrocnemius and soleus. Dengler *et al.* (1984) have found short – term synchronization in 20 of 28 pairs of human FDI motor units, and along with the findings of Dietz *et al.* (1976), it would appear that the degree of short – term synchrony is stronger in this muscle

than the cat intercostals as it was evident in short segments of data with relatively few counts, compared to the results of Sears & Stagg (1976).

Other reports of the existence of short – term synchrony in human FDI, medial gastrocnemius, tibialis anterior and biceps have appeared in abstract form (Kukulka & Bigland – Ritchie, 1980; Datta *et al.*, 1985*b*). The effects of certain pathologic conditions on synchrony have also been reported in abstract form (Datta *et al.*, 1985*a,c*; Davey *et al.*, 1986).

An assessment of the degree of synchronization of masseter motor units is necessary to validate the use of the STA technique in this muscle. There are only two reports in the literature where a synchronization test has been applied to masseter motor units. Yemm (1977*b*) looked for synchronization in 149 motor units from human masseter and temporalis muscle using the rectified EMG technique of Milner – Brown *et al.* (1973*a*) and was unable to detect any units with significant synchronization. However, this method is unsuitable for detecting tight synchronization. Goldberg & Derfler used a cross – correlation histogram on 11 pairs of human masseter single motor units and reported no tendency for synchrony, although details of the histograms were not presented. One limitation of the latter study is that the motor unit pairs used for cross – correlation were detected on the same electrode. This would underestimate the degree of short – term synchronization because superimposition of the synchronous action potentials of units recorded on the same electrode results in missed recognition of one or both units of the pair.

The available evidence suggests that the degree of synchrony in masseter motor units is small. However, both previous attempts to measure it have failed to exclude the presence of short – term synchronization, which has been suggested to be a potential source of large errors in twitch measurement using the STA technique (Kirkwood, 1979). For this reason an assessment of synchronization in masseter using cross – correlation interval histograms was performed as part of the present study. The extent of short – term synchrony in the masseter is of interest in its own right, because of the unique neural circuitry of the trigeminal muscles. The trigeminal system is believed to lack Renshaw – like recurrent inhibitory circuits (Lorente de Nó, 1947; Shigenaga *et al.*, 1988), which have been regarded as a desynchronizing influence on motoneurones (Adam *et al.*, 1978). The distribution of muscle spindle afferent synapses is also different in the trigeminal system compared to the limb muscles (Appenteng *et al.*, 1978).

A further important consideration in the planned experiments was to assess whether the degree of synchronization changed with time, as this would have an influence on the twitch fatigue data. It is generally held that synchronization increases with the duration of the contraction, or fatigue (Lippold *et al.*, 1957; Missiouro *et al.*, 1962; Person & Kudina, 1968; Mori, 1973), yet none of the methods in these experiments gave a quantitative measure of synchronization, as they all relied on the gross EMG signal. In fact, changes in synchrony with time have not been quantitatively measured for the same motor unit pairs in any muscle. This requires the use of the cross – correlation interval histogram on motor unit spike trains. Buchtal & Madsen (1950) have reported an increased tendency to synchrony after maximal contractions of 1.5 – 3 minutes duration, but did not claim to have recorded from the same pairs before and after the fatigue test. The question of the time – dependence of motor unit synchronization remains unanswered at present.

CHAPTER 2.

ASSESSMENT OF THE INDEPENDENCE OF MOTOR UNIT DISCHARGE IN HUMAN MASSETER.

2.1. Introduction.

Whenever use of the STA technique is contemplated, an important consideration is the relative independence of the trigger event to the other events producing the "noise" which it is hoped will be reduced or eliminating by averaging. For STA of motor unit twitches, this means the independence of the discharge of other motor units with respect to the trigger unit. If the discharge of motor units is not independent, their discharge is said to be correlated. A special form of correlation between motor units is synchronization, which may be defined as the tendency of a motor unit to discharge at or near the time of discharge of another motor unit. Correlated and/or synchronous activity of motor units may affect the accuracy of measures obtained using STA, and for this reason it is necessary to evaluate the extent of correlated activity, and also the likely effect of this phenomenon on the measurements obtained using STA.

Several approaches have been used in the past to assess the independence of motor unit discharge. The method presented in the original STA twitch paper used STA of the rectified and unrectified surface EMG signal (Milner – Brown *et al.*, 1973*a*; Milner – Brown *et al.*, 1975), and was also used in the present experiments as an indicator of the extent of correlated activity. However, during the course of the present work it became apparent that this method was not ideal for quantification of the extent of correlated activity, and was unable to detect certain forms of synchrony.

For this reason, I later applied a more direct and unequivocal method to detect and quantify correlated activity between motor units. This was the cross – correlation interval histogram (Moore *et al.*, 1966), in which a histogram is produced of the time of firing of other concurrently – active units with respect to the firing of a reference unit. This procedure had the advantage that it was a truly quantitative measure of motor unit synchronization, and could also be used to determine the statistical significance of any interaction found between motor units (Wiegner & Wierzbicka, 1987).

There have been two previous studies in which synchronization was assessed in the masseter as validation of the use of STA for estimation of motor unit twitches. Yemm (1977*b*) looked for synchronization in 149 motor units from human masseter and temporalis muscle with the rectified EMG technique of Milner – Brown *et al.* (1973*a*), and was unable to detect any synchronized units. In the other study, Goldberg & Derfler (1977) used a cross – correlation histogram on 11 pairs of human masseter single motor units and reported no tendency for synchrony, although details of the histograms were not presented. One limitation of the latter study is that the motor unit pairs used for cross – correlation were apparently detected on the same electrode. This would underestimate the degree of tight synchronization because superimposition of the coincident action potentials of units recorded on the same electrode results in missed recognition of one or both units of the pair.

In short, the available data suggested that synchrony of masseter motor units was likely to be weak, but the strength of synchrony had not been definitively measured in these studies. It has been suggested that even very weak synchronization, similar to that found in groups of intercostal motoneurons in the cat (Sears & Stagg, 1976), is potentially a source of large errors in twitch measurement with the STA technique (Kirkwood, 1979). The primary aim of the work presented in this Chapter was to quantify masseter motor unit synchrony, and to assess the implications of this synchrony for the use of STA. A further consideration was to determine whether the degree of motor unit synchronization changed with time, as this might affect estimates of twitch fatigue using STA. It has been asserted that the degree of synchronization increases during a prolonged or fatiguing contraction (Lippold *et al.*, 1957; Missiouro *et al.*, 1962; Person & Kudina, 1967; Mori, 1973), yet in reaching this conclusion, all of these studies inferred evidence of synchronization from the surface EMG signal. It is doubtful that any measure derived from the surface EMG gives a quantitative assessment of motor unit synchronization; the most direct and unequivocal way to measure synchrony is by cross – correlation of unit firing times. The latter approach does not appear to have been used previously to assess the effect of the duration of the contraction on the degree of synchronization of pairs of motor units.

Apart from its significance for STA, the study of synchrony of motor unit discharge has a wider relevance in helping to understand how muscles are controlled, since features of the cross – correlation histogram can be used to infer synaptic connections. In particular, a peak in the cross – correlation indicates that the cells have some common inputs (Moore *et al.*, 1970). It has been postulated

that most, if not all, motoneurones within a particular pool are likely to receive a number of common synaptic inputs (Miles, 1987). If this is the case, it should be possible to demonstrate a tendency for synchronous discharge in a large proportion of motor units within a muscle during voluntary activation.

The findings with regard to this point in the relatively few previous studies of synchronization in human motor units are contradictory. Several studies report no evidence of motor unit synchronization in a number of muscles using cross-correlation of motor unit spike trains (Kranz & Baumgartner, 1974; Goldberg & Derfler, 1977). However, other studies have found evidence of synchronization in the same, and other muscles with this technique (Dietz *et al.*, 1976; Dengler *et al.*, 1984). A limitation of all previous studies was that no test for statistical significance was applied to the synchronous peaks in the histograms. This is a serious problem if the synchrony is weak, in which case a large number of counts are required to detect it reliably in the histogram. In the present study, an attempt was made to improve on these earlier studies by the use of long recording sessions to maximise the chance of detecting weak synchrony, and also testing all peaks in histograms for statistical significance (Wiegner & Wierzbicka, 1987), in order to more reliably estimate the proportion of motor units with shared synaptic inputs.

Finally, an assessment of synchronization in the masseter is of particular interest because of the unique neural circuitry of the trigeminal system. The masticatory muscles are believed to lack Renshaw-like recurrent inhibitory circuits (Lorente de Nó, 1947; Shigenaga *et al.*, 1988), the presence of which has been postulated to have a desynchronizing influence on motor unit discharge (Adam *et al.*, 1978). In addition, the distribution of muscle spindle afferent synapses is apparently not as widespread in the masseter as is generally accepted for limb muscles (Appenteng *et al.*, 1978; Henneman & Mendell, 1981).

2.2 Methods.

Apparatus and recording procedure.

All subjects were healthy adult volunteers aged 18-40 years with normal dentitions and no history of masticatory dysfunction. All subjects gave informed consent, and the experimental procedures were consistent with the recommendations of the Declaration of Helsinki for Human Experimentation.

The subjects bit on stainless-steel bite bars with their incisor teeth. The apparatus is illustrated in Plate 2.1. The relationship of the jaws to the bars was kept constant by means of small, acrylic impressions of the subject's upper and lower incisal surfaces on the bars. The vertical separation of the bars could be altered smoothly and without backlash by turning a fine-pitch, worm-drive positioner. A similar positioner allowed antero-posterior alignment of the upper and lower bars to compensate for each subject's incisal overjet. In the experiments reported in this Chapter, the vertical separation of the incisor teeth was fixed at 6 mm for the duration of the contraction. Isometric biting force was measured by strain gauges mounted on the bars and recorded on FM tape (Hewlett-Packard 3968A, 8-channel recorder) in the bandwidth 0-1000 Hz.

Motor unit activity was recorded with two or more bipolar electrodes inserted percutaneously into the right masseter muscle. The needles were inserted deep into the muscle and spaced about 1 cm apart in a direction perpendicular to the long axis of the muscle fibres. Each electrode consisted of 3 Teflon^{R}-insulated, stainless-steel wires (70 μm core diameter) threaded through the lumen of a 26 gauge disposable needle. After insertion, the needle was removed leaving the fine wires in place. Three wires were used as this allowed the choice between 3 pairs of wires per needle insertion, and avoided the necessity of inserting a new needle if one wire proved to be faulty. The pair of wires at each needle insertion site judged to give the clearest discrimination of 1 or more single unit action potentials were used.

The surface electromyogram (EMG) of the right masseter muscle was recorded with bipolar Ag/AgCl electrodes. The skin surface was thoroughly prepared with alcohol and an abrasive paste. The gel-filled electrodes were placed about 2 cm apart near the centre of the muscle and aligned to the long axis of the muscle fibres. The muscle EMG signals were differentially amplified (Isleworth Type A101; 100-1000x) and recorded on analog FM tape (bandwidth 0-2500 Hz). In early experiments an earth electrode was attached to the subject's alcohol-prepared earlobe. In later experiments this was replaced by a lip electrode, which was simpler to use and more comfortable for the subject. Plate 2.2 shows the experimental arrangement with a subject seated with his incisor teeth on the bite bars.

Protocol.

The subject was seated comfortably with the incisor teeth on the bite bars so that he/she could observe an oscilloscope screen on which was initially displayed the

PLATE 2.1

The bite bars used for measuring the subject's isometric biting force. The bars were constructed of stainless – steel, and securely fixed to a heavy concrete and steel table, which was supported on sand and foam rubber to damp vibration from external sources. The apparatus could be raised or lowered to suit the individual subject, and the vertical angulation could be varied by $\pm$ 60°. The labelled components are:

a; acrylic impressions of the subject's upper and lower incisor teeth on the bars. This provided a secure, reproducible means of ensuring that the point of force application on the bars remained unchanged.

b; strain gauges mounted on the bars.

c; knob controlling vertical separation of the bars.

d; knob controlling antero – posterior positioning of the bars.

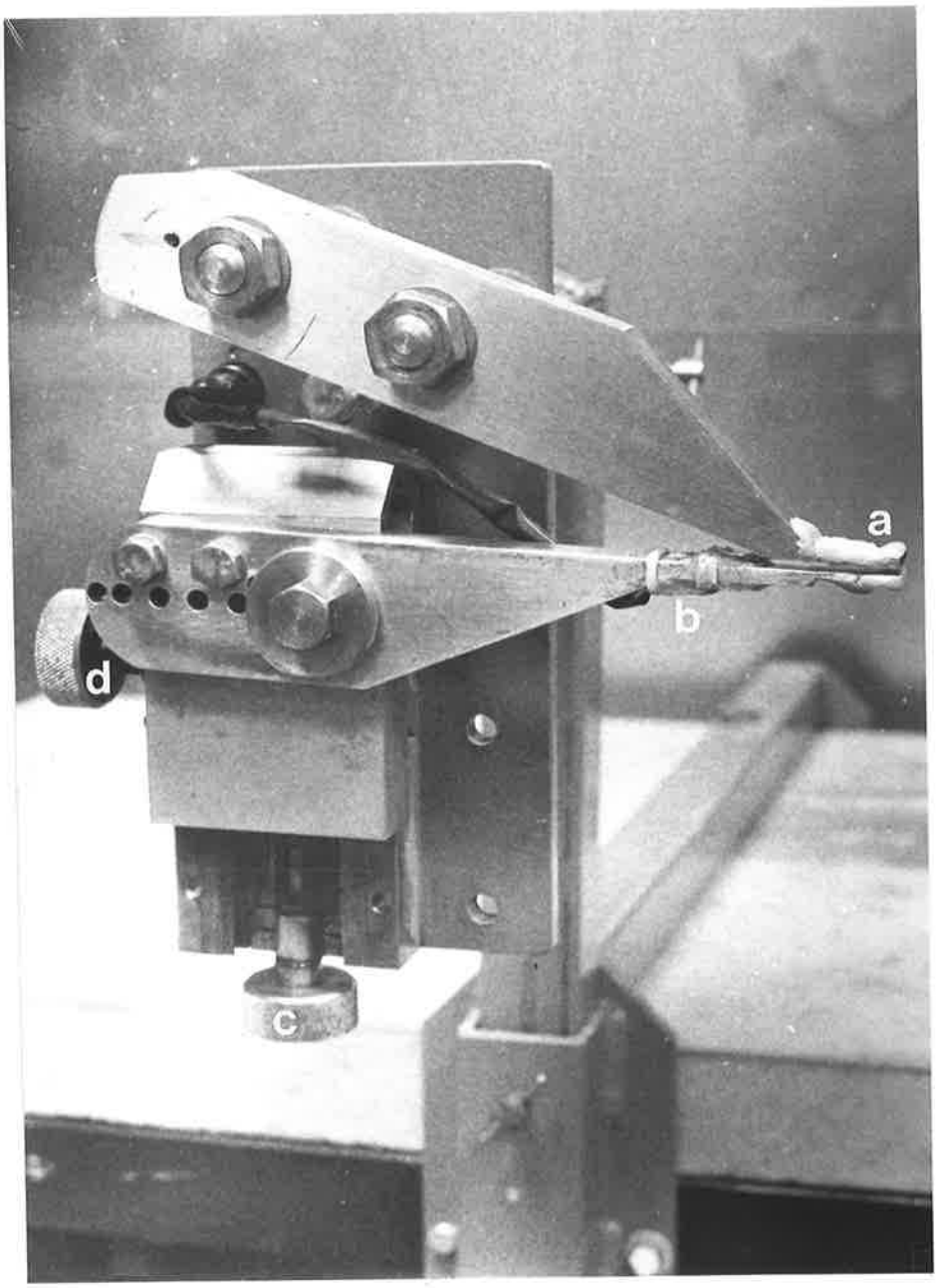

a
b
c
d

PLATE 2.2

A subject seated with his teeth on the bars. Note rotation of the apparatus so that the bars are aligned to the subject's temporomandibular articulation to allow the incisor teeth to exert force at 90° to the bars.
The labelled components are:
a; intramuscular fine – wire electrode. The needle has been retracted and taped in place, leaving the wires inserted in the muscle.
b; surface EMG electrode.
c; "lip – clip" ground electrode.


b
c
a

biting force and a ramp – shaped target line. Several slow force – ramps were tracked by the subject in order to allow off – line determination of the activation force thresholds of the active units. After the ramps, the subject was allowed to rest for 1 minute before the start of the experiment proper. The visual feedback was then switched to display the mean firing rate of one selected unit, detected in one of the 2 or 3 separate electrode sites. The subject was instructed to bite on the bars with the force necessary to keep the unit firing continuously at a mean rate of 10 Hz. In the first stage of the experiment the records from each electrode site were carefully scrutinised to ensure that action potentials from the same unit were not visible in more than one channel. This was readily achieved by displaying the intramuscular records simultaneously on an oscilloscope and triggering the sweep on the discriminable unit potentials in each channel in turn. Strongly time – locked waveforms in the other channels, (i.e. from other fibres of the same unit) were easily recognized. When this occurred, the electrodes were repositioned by pulling gently on the wires, and/or the force of contraction was varied until the offending unit was no longer seen in more than one channel, or dropped out, and one of the other remaining active units was selected to be the feedback unit controlled at 10 Hz by the subject. Once the feedback unit was selected, the subject was required to keep it continuously firing at a mean rate of 10 Hz for 15 minutes, or longer on some occasions.

In earlier experiments, motor unit potentials were discriminated on – line with a hardware amplitude – window device. In later experiments, motor unit action potentials were discriminated on – line using a computer – aided spike separation device (the SPS 8701) which used a template – matching algorithm, and was developed in this laboratory (Miles *et al.*, 1987). The subject was given visual feedback on the mean firing rate of the selected unit on an oscilloscope. In earlier experiments this was the output of a frequency meter triggered by TTL pulses from the amplitude discriminator. In later experiments, digitally – filtered mean firing rate was available as an analogue output from the SPS 8701, and this was led to the oscilloscope to provide visual feedback to the subject.

Analysis.

STA of rectified and unrectified EMG.

Off – line STA was performed on both the rectified and unrectified surface EMG signals using a computer (PDP 11/73, sampling rate 1000 Hz per channel). The averaging computer was triggered by pulses derived from motor unit potentials

discriminated with the SPS 8701, or a hardware amplitude – window discriminator. The unrectified EMG signal was filtered in the bandwidth 2 – 500 Hz before being amplified and led into one channel of the averaging computer. The EMG signal was also full – wave rectified and filtered in the bandwidth 0 – 500 Hz, before being amplified and led into another channel of the computer. The gain of both amplifiers was calibrated before each analysis session to ensure that both the rectified and unrectified EMG signals were led into the computer at identical gain. On some occasions the entire experimental trial was digitized, whereas on others only sections were analysed. STA was performed using a special – purpose averaging program. The resultant averages were plotted (Hewlett Packard 7221S), and measurements were made from the plots. Comparison of the area of the unrectified and rectified averages gave an estimate of the degree of synchronous activity of other motor units in the muscle with respect to the triggering unit (Milner – Brown *et al.*, 1973*a*; Milner – Brown *et al.*, 1975). This procedure was performed for all units for which STA of twitch force was contemplated, as a screening test for evidence of synchronization. On some occasions, when more than one unit was detected, the procedure was repeated for other concurrently – active units.

Cross – correlation interval histograms.

Analysis was performed off – line. Each intramuscular EMG record was led to a separate SPS 8701 so that the motor unit action potentials from each electrode could be discriminated simultaneously. It was possible to discriminate up to 3 different motor unit action potentials in each intramuscular record per run. The interspike intervals (measured in units of 250 μs) for each discriminated unit in each channel were stored on disk.

The disks containing the stored intervals for each unit were analysed with a computer program which generated cross – correlation histograms (Moore *et al.*, 1966) of the time of firing of other units in the muscle with respect to the firing of a reference unit. In the absence of any tendency toward correlated activity the cross – correlation histogram is flat (Moore *et al.*, 1966). When a tendency toward synchronization exists, there is a peak in the cross – correlation histogram around the time of firing of the reference unit. All histograms were constructed with 1 ms bin widths and spanned a period 100 ms before and after the firing of the reference spike (i.e. 201 bins).

Each cross – correlation histogram was plotted and examined for irregularities by eye, before analysis with a special – purpose program that assessed the strength

and statistical significance of peaks in the histogram. The most useful index of the strength of synchronous discharge of motor unit pairs was the k' value, which is the ratio of the mean bin count within the peak in the histogram to the mean bin count in the rest of the histogram. The area of the histogram over which the k' was calculated was always centred around the time of occurrence of the reference spike (time 0). A decision was required regarding the width of the peak to be included in the k' calculation, and based on the appearance of the histograms (see Results), a standardised peak width (J) of 7 bins (i.e. extending $\pm$3 ms with respect to time 0) was chosen to calculate the k' value in all histograms. For each histogram a k value was also calculated using just one bin as the peak, which was the index used by Sears & Stagg (1976). A k value was calculated for each histogram for the bin at time 0, and also the bin with the maximum counts. The k' value was preferred because it gave a more complete measure of the excess counts in the peak and was less noise – prone than the k value. Comparison of the k and k' values did give a measure of the sharpness of the peaks.

The statistical significance of peaks in all histograms was determined using a peak width of 7 ms, and the method of Wiegner & Wierzbicka (1987), a description of which is given in Appendix A. Each histogram was analysed for statistically significant peaks centred around both a) the time of occurrence of the reference spike (for J = 7, the area of interest was $\pm$3 ms), and b) the bin with the maximum number of counts. The minimum allowed mean number of counts per bin for valid statistical analysis using this method is 9, and data (either k' values or statistical significance) from histograms with a smaller mean bin count were not further considered.

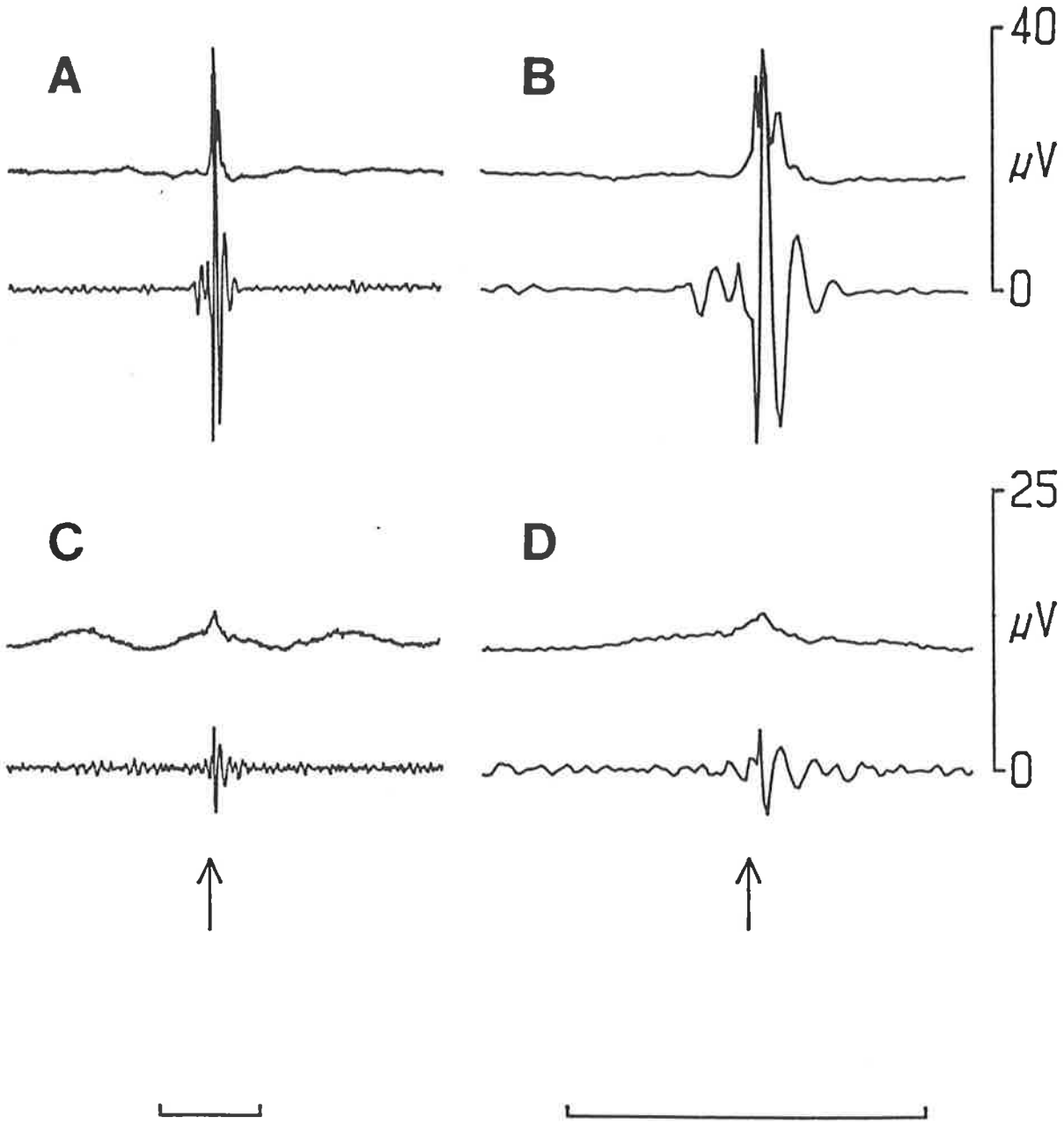
2.3 Results.

STA of rectified and unrectified EMG.

All motor units from which STA twitch measurements were contemplated were tested for synchronization using the Milner – Brown method. Fig. 2.1 shows the records from 2 representative motor units. In *A*, the average rectified EMG (upper trace) and average unrectified EMG (lower trace) are plotted on a slow time scale for a unit which showed no evidence of synchronization with this test. Both traces have the same ground potential reference point. The average rectified EMG trace is flat before and after the occurrence of the trigger spike

FIGURE 2.1

Detection of correlated activity using STA of the EMG. An example of a unit for which this test shows no evidence of correlated activity with other units is given in *A*. The lower trace is the result of averaging the EMG signal using triggers from a single motor unit. The upper trace is the average of the rectified EMG signal. Both traces are aligned with the same ground potential reference (0 mV). The same data are shown in *B* on a slower time scale. Each average in *A* & *B* is the result of 8079 trigger counts. An example of a unit exhibiting evidence of correlated activity with other units is shown in *C*, and the same data is presented in *D* on a slower time scale. Each average in *C* & *D* is the result of 7979 triggers. The time of occurrence of the trigger is indicated by the arrows. The calibration for both horizontal bars is 100 ms.


A
B
40
μV
0
C
D
25
μV
0

(indicated by the arrow), and has a sharp peak corresponding to the EMG activity of the trigger unit. This appearance indicates that in the period before and after the occurrence of the trigger spike, there was no appreciable correlated activity of other units in the muscle with respect to the trigger unit. The same data is shown with an expanded time scale in *B*, in order to show more clearly the period during which the trigger unit contributes to the surface EMG. The major component of the peak in the averaged rectified EMG above the background level is derived from the (rectified) contribution of the trigger unit to the surface EMG. This appearance has been interpreted previously as indicating the absence of synchrony (Milner – Brown *et al.*, 1973*a*; Milner – Brown *et al.*, 1975). The general pattern depicted in Fig. 2.1*A* & *B* was that found for the vast majority of units; this evidence suggested that the discharge of most masseter units was relatively independent of other units in the muscle.

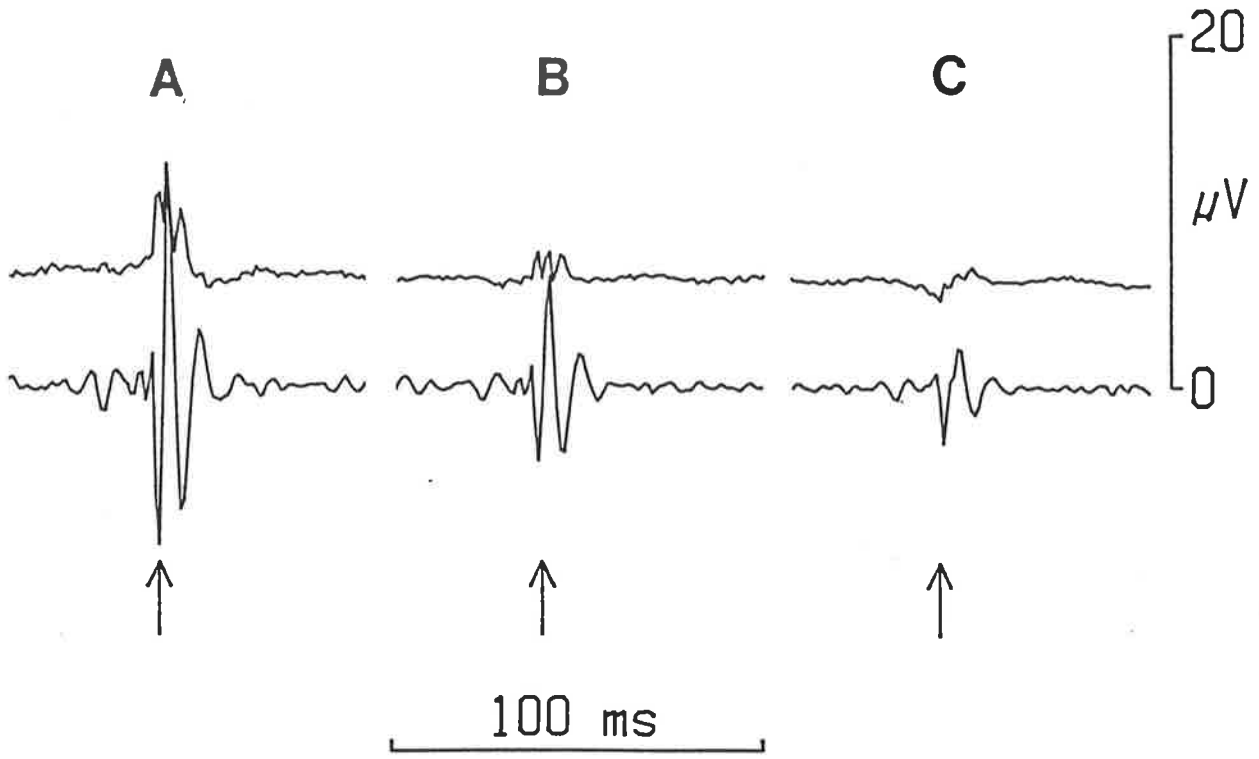
An example of a unit showing evidence of time – locked activity with other units is shown in Fig. 2.1*C*. In this example, the rectified EMG average is not flat before and after the trigger, but shows a tendency to increase in amplitude in the period 60 ms before and after it. The same data is shown on an expanded time scale in *D*. The rectified EMG average is elevated in a period in which the trigger unit does not make a contribution to the surface EMG (*C* & *D*; lowermost traces), and therefore must have arisen from the correlated activity of other units. This pattern was very uncommon, and indeed was found in only 2 masseter units. These units were excluded from STA twitch measurements.

A limitation of the rectified EMG technique is apparent in Fig. 2.1*C* and *D*. The upper and lower traces have the same ground reference point, but the contribution of the trigger unit to the surface EMG signal was not sufficiently large for it to appear above the background level in the averaged rectified EMG signal. This makes it impossible to apply the Milner – Brown criteria to estimate the extent of synchronous activity of other units with the trigger unit. In fact, in the example in Fig 2.1*C* & *D*, the elevation of the rectified EMG average in the period 60 ms before and after the trigger revealed correlated activity of other units, and so disqualified this unit from being used for STA twitch measurements, without the need to assess synchronization.

The importance of the signal – to – noise (S/N) ratio for the use of this method to assess tight synchronization is illustrated in Fig. 2.2, in which the unrectified and rectified EMG averages are shown for three concurrently – active units during the same contraction. In *A*, the unit with the largest amplitude in the intramuscular

FIGURE 2.2

The importance of the S/N ratio for detecting motor unit synchrony with STA of the EMG. The lower trace in each case is the result of averaging the surface EMG with respect to the trigger unit, and the upper trace is the averaged rectified EMG. Each trace has the same ground potential reference (0 mV). The traces in *A*, *B*, & *C* are the result of averages triggered by 3 different, concurrently – active units during the same contraction. The trigger unit for *A* was the unit controlled at 10 Hz by the subject, and of the three, this was the unit which gave the largest contribution to the surface EMG signal. The trigger units in both *B* & *C* gave a smaller contribution to the surface EMG, which did not exceed the background level resulting from the activity of all other units in the rectified EMG average. This made it impossible to use the standard criteria for assessing synchrony with this method. The time of occurrence of the trigger is indicated by the arrow below the respective traces. 1411 triggers were used in *A*, 2114 in *B*, and 3132 in *C*.


A
B
C
20
μV
0
100 ms

record was used as the trigger. As was usually the case, this was the unit controlled at 10 Hz by the subject. The rectified EMG average is flat in the period before and after the trigger, and the average contribution of this unit to the unrectified surface EMG is sufficiently large to extend above the mean rectified EMG level. Again, all traces have the same ground potential reference level. The analysis showed that most of the area of the peak in the averaged rectified EMG around the time of occurrence of the trigger was due to the trigger unit's contribution, and therefore it would be concluded that significant synchronization of other units with the trigger unit was not present. The trigger unit in *B* had a smaller amplitude in the intramuscular record, and its averaged contribution to the surface EMG was also smaller than the unit in *A*. However, since it was recorded during the same contraction, the mean total rectified EMG signal is similar to that in A, with the result that the average surface contribution of this unit does not extend above the level in the rectified EMG average due to the total unit activity. The amplitude of the average surface contribution of the trigger unit in *C* is even smaller. When the amplitude of the surface representation of the reference unit was small in relation to the total EMG activity ("noise") the conventional criteria could not be used to assess tight synchrony. These limitations of the technique, and others addressed in the Discussion, prompted me to use cross – correlation histograms to assess synchrony.

Cross – correlation histograms.

In most experiments, the feedback unit could be discriminated without difficulty throughout the duration of the contraction, which was usually about 15 minutes. The number of units which could be discriminated in the other channel(s) frequently varied during the course of the experimental run. Some units became impossible to discriminate (possibly due to small movement of the electrode or haemorrhage near the electrode tip), and, on occasion, other units appeared in the records which were distinctly different in waveform and mean firing rate to those discriminable at the onset of the contraction. For these technical reasons, it was relatively rare to identify reliably a particular unit in other channels throughout the entire 15 minute contraction. Nevertheless, cross – correlation histograms with a sufficient number of counts to allow statistical analysis of peaks in the histogram (i.e. mean bin count > 9; Wiegner & Wierzbicka, 1987) were constructed for 90 pairs of masseter motor units from 5 subjects.

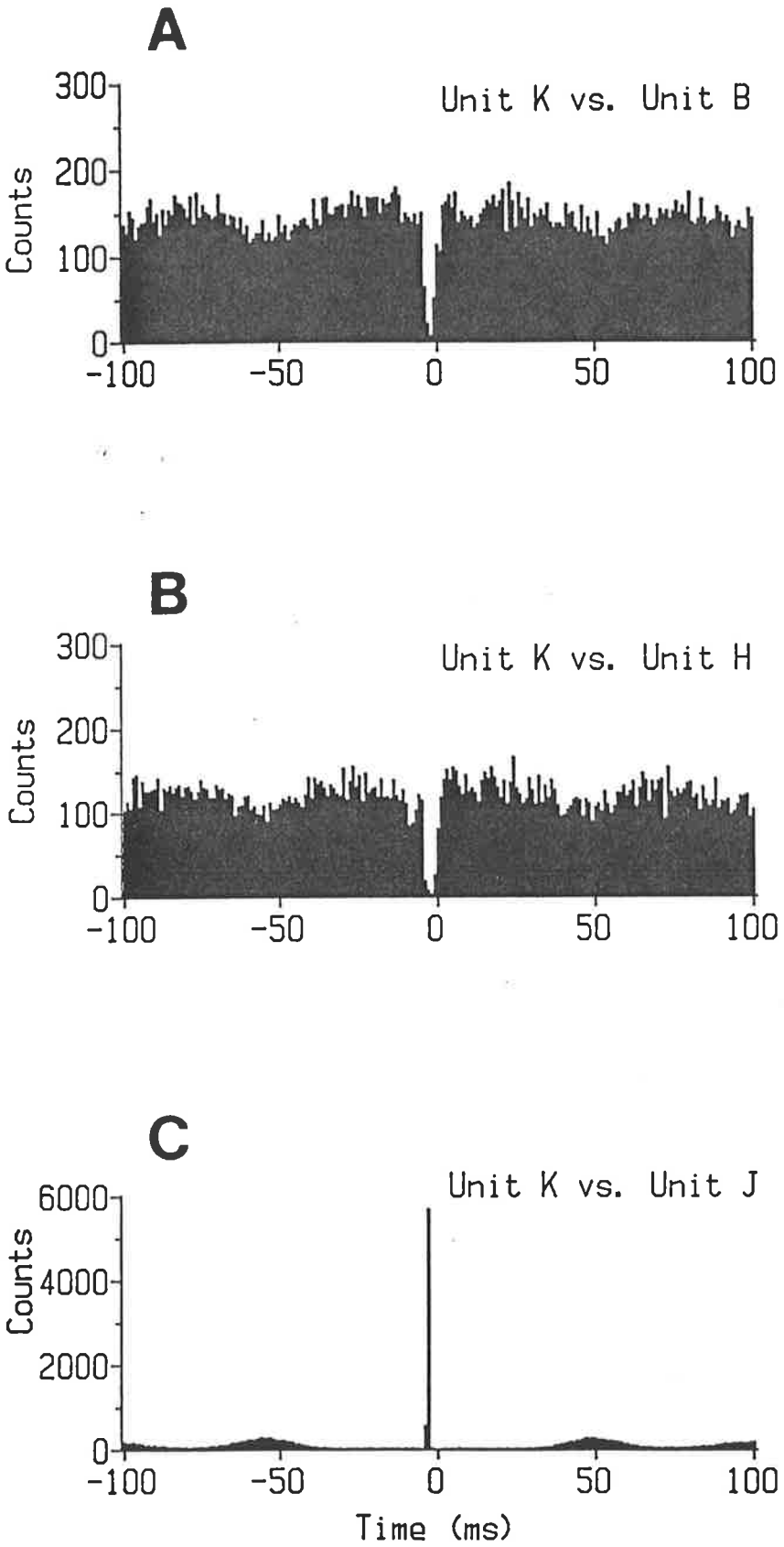
Detection and avoidance of errors influencing the cross – correlation.

In multi – unit records from the same electrode, the occurrence of action potential superimpositions make it impossible to discriminate the unit firing events with accuracy approaching 100% without very sophisticated computer techniques (e.g. Le Fever & De Luca, 1982). Although the SPS 8701 allowed greater discrimination accuracy than simple hardware amplitude – window devices, superimposition of unit potentials in the same channel still produced a false negative error of discrimination for one or both units. When the interval trains from these units detected on the same electrode were cross – correlated, a large dip in the histogram around time 0 resulted. For this reason, unit interval trains were only cross – correlated if they were recorded on separate electrodes.

Furthermore, units were also cross – correlated only if there were no units in either channel that were common to both electrodes, as this also introduces a non – random error of action potential discrimination (due to superimpositions) that distorts the cross – correlation histogram. The records in each electrode were checked for common units on – line to avoid recording useless data. On some occasions, however, this problem was not detected at the time of data collection. This was readily detectable off – line by characteristic changes in the cross – correlation histograms, and these records were then eliminated from the study. Fig. 2.3 illustrates this point with the cross – correlation histograms for 3 unit pairs (K vs B, H & J). The reference unit (unit K) for the histograms was the same in each case, and was discriminated from a separate channel to the other 3 units. The histograms in *A & B* have a sharp dip in the cross – correlation near the time of occurrence of the reference unit. In the absence of any other information this could be interpreted as evidence of correlated activity between the two units. However the histogram (*C*) produced by cross – correlation of unit K with unit J identifies the source of this correlation. This histogram has a large narrow peak near time 0, and smaller, broader peaks at about $\pm$50 ms, corresponding to the 20 Hz mean firing rate of unit J. This is the characteristic appearance of an auto – correlogram, which means that unit K and unit J are one and the same. The explanation for the large central dip in the cross – correlation histograms in Fig. 2.3*A,B* is that with a unit common to both channels, superimposition of action potentials in the second channel prevents either unit B or H firing events to be recognized and included as a count in the histogram when they coincide with a firing of unit J, which is actually the reference unit K. The broader dips at $\pm$50 ms are secondary effects due to the periodicity of unit K, as seen in the autocorrelogram (Fig. 2.3*C*).

FIGURE 2.3

The effect on the cross – correlogram of the presence of action potentials from a unit common to both electrodes. The cross – correlation interval histograms for three different unit pairs are shown in *A* – *C*. The same unit (unit K) was the reference unit in each case, and was detected on a separate electrode from the other units during the same contraction. In both *A* (unit K vs. unit B) and *B* (unit K vs. unit H) there is a sharp dip in the histogram near the time of firing of the reference spike. The histogram in *C* indicates the reason for this abnormal appearance. The large, narrow central peak (note the different vertical scale) in the histogram in *C*, the absence of counts in the period 40 ms either side of this peak, and the grouping of counts at $\pm$ 50 ms (corresponding to the firing rate of unit J) indicates that this cross – correlation is in fact an auto – correlation of unit K with itself. That is, action potentials from muscle fibres of unit K were present in both electrode channels, and were initially mistaken as arising from different units. The presence of action potentials from a unit common to both channels leads to superimposition errors of recognition which cause artifacts in the cross – correlation histograms such as those shown in *A* & *B*. In *C*, the central peak is offset several milliseconds from time 0. This was atypical, and the peak in the cross – correlation was usually found within $\pm$ 1 ms of time 0. The timing difference presumably arose from a combination of conduction differences with respect to electrode location, and whether an early or late feature of the action potential waveform was used to trigger the recognition device.


A
Unit K vs. Unit B
Counts
300
200
100
0
-100
-50
0
50
100
B
Unit K vs. Unit H
Counts
300
200
100
0
-100
-50
0
50
100
C
Unit K vs. Unit J
Counts
6000
4000
2000
0
-100
-50
0
50
100
Time (ms)

Characteristics of masseter unit cross – correlation histograms.

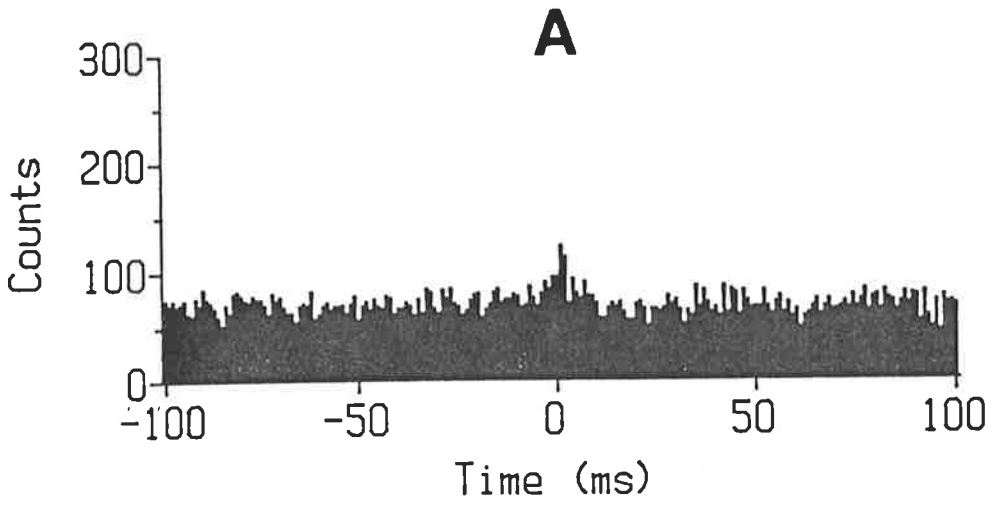
The histogram in Fig 2.4*A* illustrates the typical appearance of peaks found in masseter cross – correlation histograms. Peaks were usually centred within 1 or 2 ms of time 0, with a narrow central peak of about 3 ms in width, on a broader base. In some histograms there were no discernible peaks. An example of this is shown in Fig. 2.4*B*. The test used in the present study for determining the significance of a peak in the cross – correlation histogram required a decision about the width of the peak (J) to be analysed. Determination of the peak width is somewhat arbitrary, but it is desirable to choose an appropriate value for J based on the appearance of the peaks in the histograms. In most histograms in which a peak was present near time 0, the peak was spread over about 7 – 11 bins (ms) in which the bin count was above the mean level. A peak width of 7 ms was found to be optimal for statistical analysis of peaks in the masseter cross – correlation histograms. This value was chosen because, when the same histograms were analysed with J = 9 or 11 ms, fewer peaks were found to be significant; alternatively, using a smaller value did not increase the likelihood of a peak being deemed significant using this test. For the results presented herein, all peaks in histograms were analysed for statistical significance using a width of 7 ms.

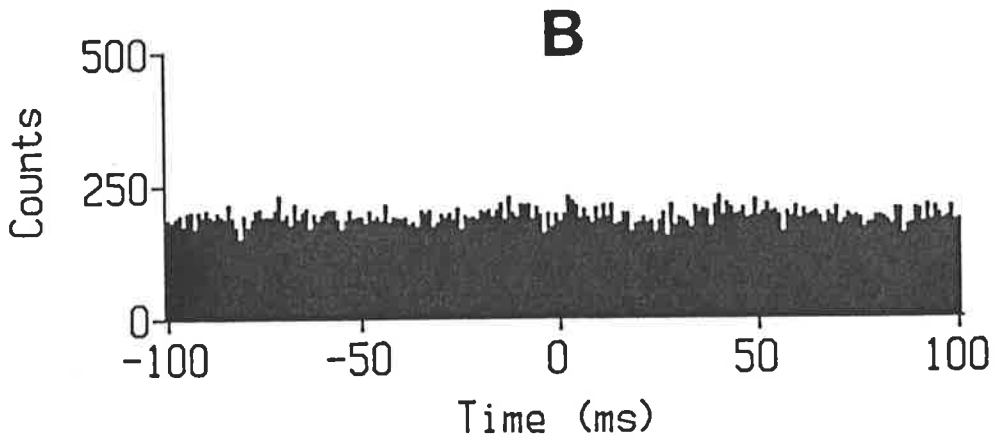
Strength and significance of synchrony.

A k' value was calculated for all unit pairs in the $\pm$3 ms region of the histogram centred around time 0. The mean k' value ($\pm$ SD) for the 90 unit pairs tested was 1.12 $\pm$ 0.12, with individual values ranging from 0.85 – 1.49. Significant peaks in the cross – correlation histogram around time 0 could be demonstrated in 45 of these 90 pairs (50%). The significance of a peak in the histogram is determined by comparison of the number of excess counts in the peak to the mean bin count in the rest of the histogram, along with the a consideration of the variance in the bin counts due to chance. The ability to demonstrate significance of a peak is related to both the strength of the correlation (given by the k' value), and also the number of counts in the histogram, as the latter affects the variance of the bin counts. The weaker the correlation, the greater the number of counts required in the histogram to identify that the correlation is significant. This point can be illustrated by reference to Table 2.1, in which the histograms have been subdivided on the basis of the total number of counts in each. Histograms grouped for analysis were those with a mean bin count between 9 and 50; those between 50 and 100; and those greater than 100. The mean strength of

FIGURE 2.4

Typical cross – correlation interval histograms from masseter unit pairs. The example in *A* is from a pair of units for which cross – correlation of their firing times produced a peak in the histogram near time 0. The k' value for this peak (±3 ms of time 0) was 1.36, and the peak was significant ($P < 0.001$). The histogram in *B* is from another pair of units, and in this case no obvious peak was detectable anywhere in the histogram. The k' value for the bins within ±3 ms of time 0 was 1.03, and this peak was not significant ($P > 0.05$).


A
300
200
100
0
Counts
-100
-50
0
50
100
Time (ms)


B
500
250
0
Counts
-100
-50
0
50
100
Time (ms)

synchrony (k') was similar for each group, and a one-way ANOVA showed no significant differences between them (Table 2.2). However, in the histograms with a mean bin count less than 50, the percentage of pairs with a significant correlation was only 36%, compared with 53% of pairs when histograms with a mean bin count between 50 and 100 were used. The percentage of significantly-correlated pairs rose to 89% in the group of histograms with a mean bin count greater than 100.

TABLE 2.1

THE EFFECT OF THE NUMBER OF COUNTS IN THE CROSS-CORRELOGRAM ON THE DETECTION OF SIGNIFICANT SYNCHRONIZATION.

mean bin count	*mean k' (± SD)*[1]	*No. pairs.*	*No. pairs with synchrony (%)*[2]
$9 < \underline{b} < 50$	1.13 ± 0.13	55	20 (36%)
$50 < \underline{b} < 100$	1.11 ± 0.13	17	9 (53%)
$\underline{b} > 100$	1.12 ± 0.07	18	16 (89%)
Total		90	45 (50%)

[1] calculated with J = 7 ms, all histograms have 201 bins.

[2] pairs in which significant peaks around time 0 were demonstrated in the cross-correlogram ($P < 0.05$).

TABLE 2.2

ANOVA TEST OF THE STRENGTH OF SYNCHRONY IN HISTOGRAMS WITH DIFFERENT MEAN BIN COUNTS.

Variable (grouping by bin count) vs. variable (k').

Source	*D.F.*	*SS*	*MS*	*F ratio*	*F Prob.*
Between groups	2	0.004	0.002	0.15	P > 0.05
Within groups	87	1.307	0.015		
Total	89	1.311			

All histograms were also analysed for a significant peak in the 7 ms region of the histogram centred around the bin with the highest number of counts. In all cases in which a significant peak was demonstrated around time 0, the bin containing the highest number of counts was within this ± 3 ms window, and statistical analysis of the peak centred around the bin with the maximum count also gave a significant result. However, in other cases in which there was no synchrony, the bin with the maximum count could occur by chance in any part of the histogram, and this was a useful control for the synchronization test. In 3 of 45 histograms, in which the maximum count was found in a bin outside of the ± 3 ms central window, analysis of a ± 3 ms segment centred around the bin with the maximum count revealed a significant peak. This is consistent with the number of significant peaks expected due to chance with this test (5%), even though the selection of the central bin of the peak was slightly biased.

As an estimate of the sharpness of the synchronous peak, a k value was calculated for each histogram. The bin chosen for inclusion in this calculation depended on the result obtained with the significance test for the ± 3 ms region of the histogram. When no significant peak was detected, the time 0 bin was used. If a significant peak had been found, the bin with the maximum count was used. This was always within the ± 3 ms region, and was usually the bin at ± 1 ms, rather than the time 0 bin, presumably because of peripheral conduction – time differences, and whether the discriminator was set to trigger on an early or late phase of the action potential, as the trigger was the reference for the time of

occurrence of the spike. The mean k value for the 90 unit pairs was 1.20 ± 0.40 (±SD).

Subject dependence.

Cross – correlation histograms for more than 5 motor unit pairs were obtained from 4 subjects. A summary of the histogram data for each subject is presented in Table 2.3. The mean k' value did not differ significantly between subjects (ANOVA test; Table 2.4), and it was therefore concluded that for the individuals tested, the strength of synchronization of masseter motor units was not subject – dependent.

TABLE 2.3

SUBJECT – DEPENDENCE OF THE STRENGTH OF MOTOR UNIT SYNCHRONIZATION.

Subject	*mean k' (± SD)*[1]	*Range*	*No. of pairs.*
S.K.	1.12 ± 0.13	0.86 – 1.49	49
D.R.	1.22 ± 0.20	0.94 – 1.47	6
K.T.	1.13 ± 0.10	1.04 – 1.36	11
M.N.	1.09 ± 0.06	0.85 – 1.17	23

[1] calculated with J = 7 ms.

TABLE 2.4

ANOVA TEST OF THE STRENGTH OF SYNCHRONY IN DIFFERENT SUBJECTS.

Variable (grouping by subject) vs. variable (k').

Source	*D.F.*	*SS*	*MS*	*F ratio*	*F Prob.*
Between groups	3	0.071	0.024	1.64	P > 0.05
Within groups	86	1.241	0.014		
Total	89	1.312			

Time dependence of synchrony.

The influence of the duration of the contraction on the strength of synchrony between pairs of motor units was examined in 16 pairs that were followed for 15 minutes of continuous activity. Cross – correlation histograms for each pair were constructed for each 3 – minute epoch during the 15 minute contraction. The mean bin count for each 3 – minute epoch histogram was checked to ensure that it exceeded 9 counts.

The k' value from the 1st 3 – minute epoch was calculated for each pair of units. The mean k' value ($\pm$SD) for the 16 pairs in the first 3 – minute epoch was 1.16 $\pm$ 0.12. The mean k' value for the same 16 pairs calculated in the last 3 – minute epoch (minutes 12 – 15 of the contraction) was 1.12 $\pm$ 0.11. The two values were not significantly different (paired t – test, $P > 0.05$), which suggests that continuous isometric contraction for 15 minutes did not influence the strength of synchrony of the motor unit pairs tested.

In another test of the time – dependence of synchrony, the k' value calculated from each 3 – minute epoch during the 15 – minute contraction was plotted against the duration of the contraction, and linear regression analysis performed. An example of the effect of the contraction – duration on the extent of synchrony between motor unit pairs is shown in Fig. 2.5. Cross – correlation histograms were constructed for each 3 – minute epoch during the contraction. In each epoch, 4 other units were concurrently – active with the reference unit, and separate cross – correlation histograms were constructed for all 4 with reference to the same unit. In Fig. 2.5, the k' value for each unit pair is plotted against the epoch in which it was calculated, and each of the four different symbols represents a different unit pair. There was always some random variation in the k' value from epoch to epoch, but in no case was there a significant tendency for the k' value to increase or decrease with time. Linear regression analysis of the k' value against the duration of the contraction was performed for the 16 unit pairs, and in no case was a significant correlation found.

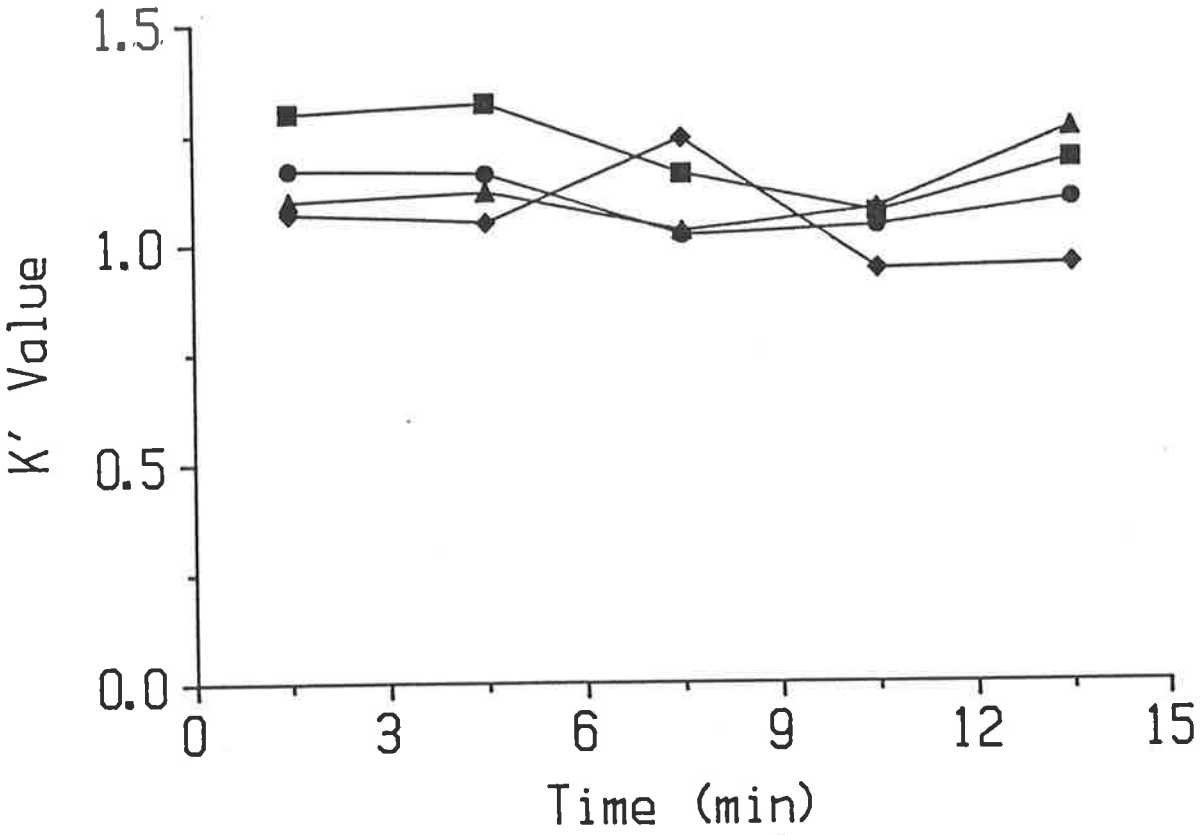
2.4 Discussion.

STA of rectified and unrectified EMG.

The usual finding with this test was no evidence of correlated motor unit activity. However, several aspects of the validity of this test need to be addressed. The test relies on the fact that with randomly distributed firings of the other units,

FIGURE 2.5

The effect of contraction – duration on the strength of synchrony between motor unit pairs. The four different symbols represent the k' value obtained from cross – correlation histograms of firing times for 4 different pairs of motor units. The reference unit was the same in each case, and the four other units were detected on separate electrodes during the same isometric contraction of 15 minutes duration. For each unit pair, a cross – correlation histogram was calculated from each 3 – minute epoch during the 15 minutes. The k' value from each epoch is plotted against the duration of the contraction for each of the pairs. Although there is some fluctuation in the epoch – to – epoch values, there was no consistent trend for the k' value to increase or decrease with time.


1.5
1.0
0.5
0.0
K' Value
0
3
6
9
12
15
Time (min)

their waveforms cancel out in the unrectified average due to temporal dispersion of the positive and negative peaks, but in the rectified EMG average all peaks summate positively to produce a flat level. Correlated unit activity with the reference unit is seen as deviation from the expected flat line offset from ground in the rectified EMG average. The most obvious correlated activity detected is the autocorrelation with the reference unit's own surface EMG contribution. This appears as a central peak in the rectified EMG average for the period in which the unit firing contributes to the surface EMG (usually about $\pm$10 ms). In the period outside of this central area, in which the reference unit does not contribute to the EMG signal, interpretation is straightforward. Any peak or trough in the averaged rectified EMG trace in this region must be due to correlated activity of other units with respect to the reference unit. With few exceptions (e.g. Fig 2.1*B*) units showed no evidence of correlated activity in this region.

The interpretation of the peak in the rectified EMG average from the period in which the trigger unit contributes to the surface EMG is more complicated. This area of the peak in the rectified EMG average above the steady mean level mean will be comprised of an element due to the contribution of the reference unit to the surface EMG, and also an element due to any correlated activity of other units. The component due to the reference unit can be determined by rectifying the unit's unrectified average contribution to the surface EMG and measuring the area of the waveform that is above the mean steady background level in the rectified EMG average. In their original paper, Milner – Brown *et al.* (1973) mathematically derived that the area obtained with this process (rectifying the average EMG contribution) would be less than the area obtained by averaging the rectified EMG contribution. In a later paper this was taken as a 20% difference, and so if the area above the steady rectified EMG average near the time of firing of the reference unit was more than 20% greater than the area of the averaged – then – rectified contribution of the unit above the steady level, the extra area was considered to be due to synchronous activity of other units (Milner – Brown *et al.*, 1975).

In many cases, when the 10 Hz – feedback unit was used as the reference unit, it gave a sufficiently large contribution to the surface EMG signal to allow the above criteria to be used to assess synchrony. However, on a number of occasions the amplitude of the reference unit's surface representation was too small to appear above the level of the rectified EMG average due to the total EMG activity (e.g., Fig. 2.2*B*,*C*), and so the method could not be used to assess

synchrony. As illustrated in Fig 2.2, this is essentially a S/N ratio problem. This problem was apparently not encountered in the FDI by Milner – Brown *et al.* (1973*a*). It is probable that the S/N ratio is superior in FDI than masseter, because FDI is a physically – small muscle with only about 120 motor units (Feindel *et al.*, 1952), compared to the much larger masseter.

There are other problems with the technique that limit its usefulness in detecting tight synchronization. The extra increment in the rectified EMG average caused by correlated units arises because the synchronous firings have a degree of temporal dispersion, so that their waveforms average out in the unrectified EMG, but remain when the rectified EMG is averaged, and can therefore be detected by comparison with the unrectified average. However, very closely time – locked synchrony of other units with the reference unit cannot be detected with this method, as the waveform in the *unrectified* EMG average of the reference unit will have an extra component due to exactly synchronous firings of the other units that cannot be distinguished. Although it is likely that synchronous discharges will have some temporal dispersion, the only way to measure very tight synchrony with confidence is by cross – correlation of unit firing times.

The rectified EMG technique, like all measures of synchrony derived from the surface EMG signal, is not strictly quantitative since each unit does not give an equal contribution to the surface EMG (Yemm, 1977*a*), and the contribution to the EMG signal may change with time independent of any change in synchronization (e.g., from slowing of muscle – fibre conduction velocity during fatigue). It also became apparent that this technique was not sufficiently sensitive to detect the weak synchronization that could be demonstrated by cross – correlation of unit firing times, but which had been implicated as a possible source of large errors in twitch measurement with STA (Kirkwood, 1979).

For these reasons, this technique was not considered to be acceptable for quantitative measurement of motor unit synchronization. However, since it samples the tendency to synchronous discharge of a large proportion of the motor unit population with respect to the reference unit in a single test, it was valuable as a screening procedure in order to exclude units from STA twitch measurement when obvious correlated or synchronous activity with other units was evident, as in Fig 2.1*B*. For quantitative assessment of motor unit synchronization it was necessary to use cross – correlation histograms.

The influence of errors in action potential discrimination.

The ideal situation is to discriminate motor unit action potentials with 100% accuracy. This is readily achievable if action potentials from only one unit are visible in each channel. In practice, however, this is rarely the case, and usually the action potentials from several motor units are detected on each electrode. With multi – unit recordings from the same electrode the SPS 8701 (or hardware amplitude – window discriminators) could not detect *all* firings of each unit because of superimposition of action potentials (i.e., false negative errors occurred). This is only feasible with highly sophisticated computer techniques involving very time – consuming off – line analysis (e.g., Le Fever & De Luca, 1982). In contrast to the hardware devices, the SPS 8701 was able to discriminate motor units with very few false positive errors by appropriate adjustment of the tolerance for the matching of an action potential with its template. In other words, few spikes were wrongly classified with the SPS 8701, although some unit firings were not recognized in multi – unit records.

The recognition error due to superimposition of action potentials will influence the cross – correlogram if the superimpositions are non – random with respect to each other. Clearly this is the case when both units to be cross – correlated are detected in the same channel, because all superimposition errors occur at the time of firing of the reference unit. Early studies apparently overlooked this source of error (Kranz & Baumgartner, 1974; Goldberg & Derfler, 1977), but most later studies have cross – correlated units only from separate channels.

However, this precaution alone does not ensure valid cross – correlograms from multi – unit recordings. As demonstrated in Fig 2.3, the presence of action potentials from different fibres of the same motor unit in both channels can also render the cross – correlation useless. The fibres from a given motor unit extend over a wide territory (Edström & Kugelberg, 1968), and recording from fibres of the same unit with intramuscular electrodes separated transversely by at least 1 cm was not an isolated incident in the masseter. In the present study these instances were identified, and the data eliminated from further consideration.

What is the effect on the cross – correlation of recognition errors due to superimposition if neither channel has common motor unit potentials? There would be no effect on the cross – correlation if all units fired independently, since the superimpositions of various units would be randomly distributed. However, if

units have a tendency to synchronous discharge, the effect of non-recognition (due to superimpositions) would be to slightly reduce the observable peak in the cross-correlogram. This can be explained as follows: Assume that all units in this muscle have a tendency to synchronous discharge, with the result that the probability of a coincident spike in any 2 units is increased by 1/100 (this is about the strength of synchrony in masseter units calculated from the cross-correlograms, see Section 2.5). Given this, a cross-correlation between unit A in one channel and unit B in another, would yield an observed correlation of this strength, provided A and B were the only units present in each channel. However, if there is also another unit C in the second channel which introduces a recognition error due to superimposition, then the observed increased probability for a coincident A & B, or A & C spike from the cross-correlation will not be 1/100. It will be reduced for each by the increased probability of A, B & C all being coincident; in this case 1/100 x 1/100 or 1/10,000. An error of approximately this magnitude will be additive for each additional unit in each channel. In the present study this error was ignored, as it remains insignificant unless a large number of unit potentials are present in each channel.

Masseter Unit Synchronization.

The results show that masseter motor units do not fire completely independently of each other, but rather have a weak tendency for synchronous discharge. The pattern of synchrony observed in the masseter units was similar to the synchrony observed by Sears & Stagg (1976) between groups of intercostal motoneurons in the cat responding to voluntary synaptic drive, and which they termed "short-term synchronization". This type of synchrony was attributed by them to be due to "the joint occurrence of unitary excitatory post-synaptic potentials (EPSPs) evoked in motoneurones by branches of common stem pre-synaptic fibres". Support for this hypothesis was provided in a later paper in which common transient depolarizations (due to common EPSPs) in cat intercostal motoneurons were detected using STA (Kirkwood & Sears, 1978).

The ability to recognize this weak synchrony in cross-correlation histograms is dependent on the total number of counts in the histogram. In the present study, when histograms with a mean bin count of greater than 100 were examined (i.e., more than 20,100 total counts), significant peaks were demonstrated for 16 of 18 (89%) masseter unit pairs. The k' values for the 2 non-significant peaks in this group were 1.02 and 1.03, which suggested that significance could have been demonstrated for these pairs if more data had been collected.

The conclusion from these data is that common synaptic inputs (EPSPs and/or IPSPs) are present in most, and perhaps all, masseter motoneurones. The effect of common IPSPs in the cross – correlation is to produce a broader central peak than common EPSPs (Moore *et al.*, 1970). The relatively narrow peaks in the masseter unit cross – correlation histograms suggest that their predominant synchronizing influence is from common EPSPs. This common input could be from a combination of central and peripheral (e.g. muscle spindle afferent) sources. There is evidence from several muscles that cortical neurones project widely within the motoneuronal pool (reviewed by Miles, 1987), and this could be a source of synchronization. The demonstration of a high proportion of motor units with significant synchronization, and hence common inputs, in the masseter is of interest because the distribution of muscle spindle afferent connections is not as widespread among masseter motoneurones as is generally found for limb muscles (Appenteng *et al.*, 1978). Also, the trigeminal system lacks Renshaw cells (Lorente de Nó, 1947; Shigenaga *et al.*, 1988). Although Renshaw cell recurrent inhibition has been suggested to have a desynchronizing influence on motoneurones (Adam *et al.*, 1978), this may not be the case, since common inhibitory inputs can also produce synchronization in the cross – correlogram (Moore *et al.*, 1970).

Several previous studies have demonstrated short – term synchrony in human motor units. Dietz *et al.* (1976) found narrow peaks in the cross – correlograms in 70 of 87 pairs (80%) of units from human FDI, gastrocnemius and soleus. Dengler *et al.* (1984) found short – term synchronization in 20 of 28 pairs (71%) of human FDI motor units. Both of these studies used relatively short segments of data, with only a fraction of the total counts of histograms used in the present study. Even though the strength of synchrony in the FDI is apparently stronger than that observed in the masseter (compare with Fig. 1 in Dengler *et al.*, 1984), it is likely that a higher proportion of units with synchronous discharge would have been demonstrated in these studies had they recorded an amount of data comparable to the present study. Other reports of the existence of short – term synchrony in human FDI, medial gastrocnemius, tibialis anterior and biceps have appeared in abstract form (Kukulka & Bigland – Ritchie, 1980; Datta & Stephens, 1980; Datta *et al.*, 1985). The inability of several previous studies to detect motor unit synchronization appears to be a result of discrimination of units for the cross – correlation from the same electrode, without consideration of the effects of the errors induced by action potential superimpositions (Kranz & Baumgartner, 1974; Goldberg & Derfler, 1977). Therefore, a high proportion of motor units in many

muscles exhibit short – term synchronization. This observation can now be extended to include the masseter muscle, which is innervated by a cranial nerve with its motoneuronal cell bodies in the brain. It appears to be a general principle of motor control that most, and perhaps all, motoneurones in a pool receive some common (probably excitatory) synaptic inputs.

Subject Dependence of Synchrony

Using the rectified EMG technique, several previous studies in FDI found that some subjects exhibited a greater tendency to synchronous motor unit discharge, which was apparently accentuated by a usage pattern of the muscle that involved regular high – force contractions (Milner – Brown *et al.*, 1973*a*; Milner – Brown *et al.*, 1975). With cross – correlation histograms in the present study, no difference was found in the strength of synchrony of masseter units in different subjects. It was also rare to find any evidence at all of synchrony in masseter with the rectified EMG technique. It is concluded that the strength of synchrony in masseter is not strongly subject – dependent.

Changes in Synchrony with Time or Fatigue.

The notion that synchronization increases with the duration of the contraction or fatigue, appears to have been generally accepted (Bigland – Ritchie, 1981*b*; De Luca, 1984), yet there is no compelling evidence for this conclusion. Evidence of increased synchrony during prolonged contractions has been inferred from the appearance of grouping of motor unit activity (Lippold *et al.*, 1957) and the development of large periodic oscillations in the EMG signal (Missiouro *et al.*, 1962; Mori, 1973). These changes in the EMG signal may arise by chance, because of the tendency for the motor units to fire at similar rates (Taylor, 1962). Any method of estimating synchronization from the EMG signal is not quantitative, since the contribution of different units to the signal does not summate linearly, and may change with time independent of synchronization. Cross – correlation of unit firing times is the method of choice to investigate this question, as it is an objective measure of synchronization. Buchtal & Madsen (1950) used a form of cross – correlation of unit spike trains, and did report an increase in synchrony following a maximal contraction of 1.5 – 3 minutes duration, although they did not claim to have recorded from the same units before and after the fatigue test. In the present study, with cross – correlation of firing times of the same unit pairs, there was no evidence for a significant change in motor unit synchronization during a continuous isometric contraction for 15 minutes. Evidence is presented in Chapter 5 that this type of contraction

frequently induced fatigue in the masseter units. The suggestion that motor unit synchronization increases with contraction – duration or fatigue is not supported by the results in the present study. Further studies using cross – correlation of unit firing times are indicated in other muscles, and with different fatiguing paradigms, in order to settle this issue.

2.5 The implications of the observed pattern of synchronization for spike – triggered averaging.

The results in this Chapter show that masseter motor units do not fire completely independently of each other, but rather that most units have a weak tendency to synchronous discharge. An important question is the extent of the error induced by this tendency to synchronous discharge when STA is used to determine properties of motor units such as their twitch tensions, or their contribution to the surface EMG.

For motor unit twitches.

In Section 2.3 the mean k' for 90 masseter motor unit pairs was found to be 1.12, with the peak in the cross – correlation histogram extended over $\pm$3 ms. In the following discussion, this value will be used as an indicator of the overall average degree of synchrony of masseter motor unit pairs.

Consider the simplest case, in which motor unit A is the trigger for the averaged twitch, and a second motor unit B is also active. In the STA context, these excess spikes over and above the number expected by chance will result in a force contribution from unit B that is not random with respect to A, and thus will not be completely eliminated by averaging. The timing of the excess B spikes means that the force contribution from these excess counts will appear as a positive error in the determination of the twitch force of unit A. The extent of this error can be estimated in the following way (after Kirkwood, 1979). With 1 ms bin width, and assuming for the sake of argument a mean firing rate for unit B of 10 Hz, then the expected ratio of B spikes in any bin per total number of A spikes is 0.01. The total number of extra B spikes per A spike above the expected value is given by the area of the excess peak in the cross – correlation histogram i.e. 0.12 x 7 x .01 = 0.0084. The twitches associated with these extra 0.008 B spikes per A spike synchronized to within $\pm$3 ms summate at the tendon and lead to error in the estimation of twitch amplitude, duration and rise time for unit A. If these are the only 2 units active, and give equal size twitches, then the error amounts to only 0.8%. However, in a voluntary contraction of moderate force, there will be many active units, and the synchronous firings of each will contribute to error in the twitch measurement. It has been estimated that approximately 50 masseter units are active during an incisor bite of 7 N (Miles & Türker, 1987); at higher biting forces even more will be active. If 50 units were active (assuming 10 Hz rate for each), and each had an equal size twitch, the

error in a single unit twitch measurement using STA would be 50 x 0.8% = 40%. This was the argument used by Kirkwood (1979) in his estimation of the effects of synchronization on the twitch obtained by STA, and which led him to conclude that weak short – term synchrony would induce large errors in the twitch obtained using STA.

However, in his argument, Kirkwood overlooked several important properties of motor units activated in voluntary contractions, with the result that he has over – estimated the error in STA twitches due to this degree of synchrony. Firstly, different motor units within the same muscle exhibit a wide range of twitch tensions, and are recruited in voluntary contractions in order of increasing size (Henneman & Mendell, 1981). STA of motor unit twitches requires that the trigger unit fire at a rate just above its tonic threshold, which means that the trigger unit is one of the last – recruited active units. This means the trigger unit in STA is always one of the largest active units. There is a wide range of twitch tensions for motor units within a muscle, and the distribution of motor unit twitch tensions is skewed so that the smaller – twitch units comprise the greater proportion of the active units at any level of voluntary contraction (Burke, 1981*a*). Because most of the active units are considerably smaller than the trigger unit, the mean error from each active unit's extra synchronous firings amounts to considerably less than 0.8% of the trigger unit's twitch.

The firing rate of the synchronous units also needs to be considered for two reasons. On the one hand, if synchronous units are firing faster than the trigger unit, there will be more extra synchronous firings per trigger spike, and hence a greater error. In simple terms, if a synchronous unit contributes a 0.8% error to the trigger unit twitch when both are firing at 10 Hz, the error will be 1.6% if the synchronous unit were firing at 20 Hz, and the trigger unit remained at 10 Hz. However, the mechanical properties of motor units minimise this error. At faster firing rates the twitches become progressively more fused, until a rate is reached at which a twitch can no longer be detected because the unit's force output is fully fused. Synchronous units firing at a rate sufficient for full tetanus would have *no effect at all* on the STA twitch of the trigger unit.

In order to assess the net effect of the firing rate of the active units on the STA twitch error some information is required regarding the distribution of motor unit firing rates during a voluntary contraction. The firing properties of the masseter units during these experiments are considered in Chapter 9. An indication of the distribution of firing rates of other active masseter units (background units) when

one unit (the feedback unit) is controlled at 10 Hz can be gained by reference to Fig. 9.5*A*. In the sample of background units reported in Chapter 9, 61% had a firing rate greater than 13 Hz, and 30% were firing at more than 15 Hz. Only 14% were firing at 10 Hz or less. The actual distribution of firing rates of all active units in this situation is undoubtedly more heavily weighted towards higher rates than is apparent in this Figure, because of sampling bias in the selection of background units included for analysis in Chapter 9. The inclusion of a background unit was biased towards units with larger amplitude extracellular action potentials, in order to satisfy limits imposed for the accuracy of motor unit action potential recognition and the requirement that the unit's activity be identified for 15 minutes of continuous activity. Units with larger amplitude action potentials tend to have a higher recruitment threshold (Goldberg & Derfler, 1977), and hence are more likely to have a recruitment threshold similar to the feedback unit. They are therefore more likely to be firing at a rate close to 10 Hz than units with a smaller action potential amplitude detected on the same electrode. Background units with action potentials of small amplitude which did not satisfy the stringent criteria for inclusion in Chapter 9 were frequently observed in the intramuscular records. These units were usually firing at high rates, with most estimated at greater than 15 Hz. Prolonged tonic firing at rates higher than 25 Hz have been reliably measured for masseter motor units in this laboratory, but this requires particularly fortunate electrode conditions, and it would rarely be possible to reliably discriminate such units in a multi – unit recording.

The masseter units with firing rates between above 15 Hz are therefore under – represented in Fig. 9.5*A*. I estimate that approximately two small amplitude units (firing at >15 Hz) were rejected for each background unit included in the distribution shown in Fig. 9.5*A*. At the higher range of total biting forces, a greater proportion of background units were rejected. Using this information, a more accurate estimate of the distribution of background unit firing rates would be that more than 76% of the active units fire at rates above 15 Hz. The twitches of masseter motor units are likely to be highly fused at rates above 15 Hz (Calancie & Bawa, 1986; also Chapter 3), and so units firing at these rates or higher can be ignored as a source of error in the STA twitch estimation.

It is estimated that about 5% of the active units fire at 10 Hz or less. These units would be about the same size and state of fusion as the trigger unit and thus each would contribute about a 0.8% error to the STA twitch estimation of the trigger unit. With 50 active units, this amounts to an error of 2%.

About 19% of active units are estimated to be firing at 10 – 15 Hz, with the majority of these greater than 13 Hz. Synchronous firings of these units will have some effect on the STA twitch, but the error will be less than 0.8% per unit because these units will be earlier – recruited and hence smaller than the trigger unit, and also because of the rapid increase in fusion at rates even slightly above 10 Hz. With 50 active units the STA twitch error for the trigger unit due to synchronization in this population of units is estimated at about 3%.

This amounts to an estimated absolute error in the STA twitch due to synchrony of about 5%, with 50 active units (compared with 40% using Kirkwood's original estimation). The error will be greater if there are more active units, but is unlikely to summate linearly because with a range of possible firing rates beyond 25 Hz, at higher forces a greater proportion of active units would be expected to be firing above 15 Hz. It is likely that few contractions in the present series exceeded a total of 100 active masseter units.

An error of this magnitude in the absolute twitch amplitude has a very small influence on the twitch fatigue index, because this parameter is a ratio of the initial and end twitch amplitudes, and most of the synchronization – induced error cancels out, because synchronization did not change significantly with time in the masseter units. A change in the twitch force of the synchronized units with time would have only a very small effect on the trigger unit's fatigue index. For example, the twitch force output of each background unit contributing some synchrony – related error to the trigger unit's STA twitch would need to change 20% in magnitude (each in the same direction) to produce a 1% error in the trigger unit's fatigue index. The size of the synchronization – induced error, and the inability to demonstrate a significant difference in the strength of motor unit synchronization with time suggests that synchronization can be ignored as an important source of error in twitch fatigue estimation using STA in human masseter.

For the surface representation of the unit.

The averaged surface representation waveforms of concurrently – active units were similar, except for differences in amplitude (e.g., Fig. 6.6). The waveforms typically had positive and negative peaks separated by about 4 – 5 ms. Unlike the situation with motor unit twitches, temporal dispersion of the synchronous firings over $\pm$3 ms results in phase differences in the summated EMG waveforms so that increments due to many of the extra synchronous firings cancel each other out in the final surface representation. For this reason, only the extra synchronous

firings at time 0 need be considered as a major source of error in the surface representation of the unit. The best indicator of this is the value k calculated from only the central bin of the peak, which for the masseter units in the present study was 1.20. For a unit firing at 10 Hz, this represents 0.2 x 0.01 = 0.002 or about 0.2% extra spikes synchronized with the trigger unit which could affect the surface representation waveform. The magnitude of the error in amplitude estimation of the trigger unit's surface representation will be greater if more units are active. If there are 50 units active, this gives an error of 50 x 0.2%, or 10%.

This figure should be modified, if necessary, by taking into account the size distribution of the motor units, and their surface representations. If the trigger unit is the 10 Hz – feedback unit, then it is also likely to give a larger contribution to the surface EMG than earlier recruited units (Yemm, 1977*a*) primarily because higher – threshold units have larger extra – cellular action potentials (Goldberg & Derfler, 1977). This would tend to reduce the relative error. On the other hand, the lower – threshold units in the masseter fire faster at any level of voluntary drive, and this would tend to increase the relative error, as there would be more extra synchronous firings per trigger spike. It is likely that these opposing influences balance out in the masseter, as it was found that small, faster – firing units and the large, slower – firing units during a voluntary isometric contraction of the masseter, gave similar increments to the total EMG signal over an equal period of time (Miles & Türker, 1987).

This leaves an estimated 10% error in surface representation amplitude for a trigger unit firing at 10 Hz during a voluntary contraction with a total biting force of 7 N, assuming 50 units are active. There is indirect evidence which suggests that the actual error due to synchronization is smaller than this, because a large change in the total masseter unit activity (and hence total synchronous activity) during the contraction was not accompanied by a significant change in the averaged surface representation amplitude (Chapter 6; Fig. 6.5). The inability to demonstrate significant changes in motor unit synchrony with time under the conditions of this study, and the apparent insensitivity of the averaged surface representation waveform to large changes in the total activity level of the muscle, suggests that the "noise" in the STA technique due to synchronization should not be so large as to obscure meaningful time – dependent changes in the surface representation waveform.

CHAPTER 3

THE EFFECT OF MOTOR UNIT FIRING PATTERN ON TWITCHES OBTAINED BY SPIKE – TRIGGERED AVERAGING.

3.1 Introduction

The technique of spike – triggered averaging (STA) has been widely used as a means of extracting the twitch profile of a single motor unit from the total tension during a voluntary contraction in humans (Stein *et al.*, 1972; Milner – Brown *et al.*, 1973*a*,*b*; Yemm, 1977*b*; Stephens & Usherwood, 1977; Goldberg & Derfler, 1977; Monster & Chan, 1977; Dengler *et al.*, 1988). A limitation of the technique is that the unit must be firing tonically, in order to avoid contamination of the twitch from synchronous activation of other units which inevitably occurs during phasic activation. The mean firing rate of the units during estimation of the twitches by STA is commonly 7 – 10 Hz. This constraint means that the twitches derived by STA are in fact partially fused, compared with conventional motor unit twitch measurement during animal experiments where single motor units are stimulated at a rate of 1 Hz or less (see Burke 1981 for review).

The degree of fusion of motor unit twitches obtained by STA during voluntary activation at firing rates just above the threshold for tonic firing was originally thought to be minimal, and it was suggested that the twitch profile was similar to that found with low rates of activation. This conclusion was supported by the similarity of STA twitches to those obtained by direct intramuscular microstimulation at less than 2 per second of (presumably) single units in the same region of the muscle (Milner – Brown *et al.*, 1973*a*). Further support for minimal fusion at these rates of voluntary activation came from observations that varying the firing rate of units between 7 and 10 Hz in the human first dorsal interosseous (FDI) (Milner – Brown *et al.*, 1973*a*), and 8 – 15 Hz in masseter (Yemm, 1977*b*), had little effect on the STA twitch. In contrast, Monster & Chan (1977) showed an example of fusion in single unit twitches obtained by STA in human extensor digitorum communis muscle when the mean firing rate of the unit was increased from 8 to 16 Hz.

Evidence that twitches derived by STA were likely to be significantly distorted due to fusion was presented in a critique of the STA technique by Calancie & Bawa

(1986). They studied the effect of activation rate on the twitch profile of single motor units of varying contractile speeds in cat hindlimb muscles by electrically stimulating isolated ventral – root filaments. They showed that as the stimulation rate increased, there were marked reductions in the amplitude, contraction time, and relaxation time of the twitches that were extracted by STA. The slower the contraction time of the unit, the more severe was this distortion of the true twitch (i.e. the twitch produced by stimulation at 1 Hz) as the stimulation rate increased.

These results cast some doubt on the validity of the earlier conclusions from the human work, although it is difficult to compare directly the situation of stimulating one unit in isolation in the muscle with the situation during voluntary contraction, where many units are active. The aim of the present study was to perform a systematic evaluation of the effect of the firing pattern of the unit on the twitch profile obtained by STA during voluntary contractions of human masseter. A major improvement over the earlier human work was produced by a computer program which imposed rigorous constraints on the pre – and post – spike firing activity of each spike accepted as a valid trigger for the average, so that the data were analysed with a degree of precision analogous to that of stimulation of single units in animals.

3.2 Methods.

Apparatus and recording procedure.

All subjects were healthy adult volunteers aged 18 – 40 years with normal dentition and no history of masticatory dysfunction. All subjects gave informed consent, and the experimental procedures were consistent with the recommendations of the Declaration of Helsinki for Human Experimentation.

The subjects bit on stainless – steel bite bars with their incisor teeth. The relationship of the jaws to the bars was kept constant by means of small, acrylic impressions of the subject's upper and lower incisal surfaces on the bars. The separation of the incisor teeth was fixed at 6 mm for the duration of the contraction. Isometric biting force was measured by strain gauges mounted on the bars. The biting force signals were recorded on 2 FM tape channels. In the first channel the total biting force was recorded in the bandwidth 0 – 1000 Hz. The signal in the second channel was the high – gain force record suitable for determining the twitch force of the single motor units. This was obtained by

high – pass filtering ($\tau = 1$ sec) of the total biting force to remove the DC offset, and then amplification prior to recording.

Motor unit activity was recorded with a bipolar electrode inserted percutaneously into the right masseter muscle. The electrode consisted of 2 Teflon^R – insulated, stainless – steel wires (70 μm core diameter), threaded through the lumen of a 26 gauge disposable needle. After insertion, the needle was removed leaving the fine wires in place.

The surface EMG of the right masseter muscle was recorded with bipolar Ag/AgCl electrodes placed about 2 cm apart near the centre of the muscle and aligned to the long axis of the muscle fibres. The EMG signals were amplified (1000x) and recorded on analog FM tape (bandwidth 0 – 2500 Hz).

Protocol.

The subject was seated comfortably with the incisor teeth on the bite bars so that he/she could observe an oscilloscope screen on which was displayed the mean firing rate of the selected single unit, and the subject was instructed to bite isometrically on the bars with the force necessary to keep the selected unit firing at a mean rate of 10 Hz throughout the recording period.

Analysis.

The analysis was performed off – line from the taped records. Trigger pulses for the averager were generated by real – time discrimination of single motor units using the SPS 8701. The interspike intervals (ISI's) in the train of spikes from each unit were stored on floppy disk. The trigger pulses, the high – gain force record (bandwidth 1 – 500 Hz), the surface EMG (filtered 2 – 500 Hz), and the full – wave rectified surface EMG (filtered 2 – 500 Hz) were digitised on separate channels of a PDP 11/73 computer (sampling rate 1000 Hz per channel), and stored on hard disk for analysis with the averaging program. In Chapter 4, comparison of twitches obtained with the filtering procedure used in the present study and those obtained without filtering showed that this filtering did not distort the twitch waveform.

Spike – triggered Averaging.

The general objective was to examine the effect of the pre – trigger ISI on the twitch obtained by STA of the biting force. This was determined off – line by a special – purpose averaging program. Each discriminated pulse in the train from a voluntarily – active single unit was screened to decide whether it would be

accepted or rejected as a valid trigger for the average. The 4 criteria which each action potential (or "spike") had to satisfy before being accepted as a valid trigger for the computer average were as follows:

i) There was a minimum interval (specifiable) before the spike during which the unit did not fire. This parameter set the upper limit of the acceptable instantaneous firing rate of the unit for a valid trigger.

ii) There was a larger interval (specifiable) before the trigger spike in which the unit fired at least once. This set the lower limit of the acceptable instantaneous firing rate of the unit for a valid trigger.

iii) There was a minimum interval (specifiable) after the trigger spike in which the unit did not fire. This ensured that the resultant twitch was completed before the next firing of the unit so as to avoid distortion of the twitch due to summation.

iv) There was a larger interval (specifiable) after the spike in which the unit fired at least once.

For the main part of this study the post – spike firing parameters were kept constant, and the pre – spike firing parameters for a valid trigger spike were systematically varied in order to examine the effect of the immediate firing history of the unit on the twitch obtained by STA. The same epoch of data was analysed repeatedly with different spike parameters for the trigger spikes. For each separate average, the duration of the pre – trigger interval in which no other firing of the unit was allowed (the value specified in i) above) was progressively increased in 10 ms steps. The value specified in ii) above was always 10 ms greater than the value specified in i). This ensured that the unit fired at least once within a 10 ms "window" at a specifiable interval prior to the trigger spike, thus giving precise control over the firing pattern of the trigger spikes used in each average.

In order to facilitate comparison with previous human studies of STA twitch fusion, the data were also analysed with spike parameters that emulated the less – rigorous rate limitation used therein. The parameters used were:

i) 300 – 30:30 – 300. These parameters effectively allow all spikes to be accepted as valid triggers for the average, and are equivalent to no rate limitation other than the subject's ability to control the unit at the prescribed rate (e.g. Yemm, 1977*b*).

ii) 300 – 100:100 – 300. Trigger spikes limited to those spikes with an instantaneous firing rate between 3.3 and 10 Hz.

iii) 300 – 140:100 – 300. Trigger spikes limited to those spikes with an instantaneous firing rate between 3.3 and 7 Hz. The parameters in ii) and iii) are analogous to the rate limitation used by Milner – Brown *et al.* (1973*a*).

iv) 300 – 100:30 – 300. These parameters select spikes as triggers for the average on the basis of the pre – trigger firing interval alone (i.e., effectively all post – trigger intervals were acceptable).

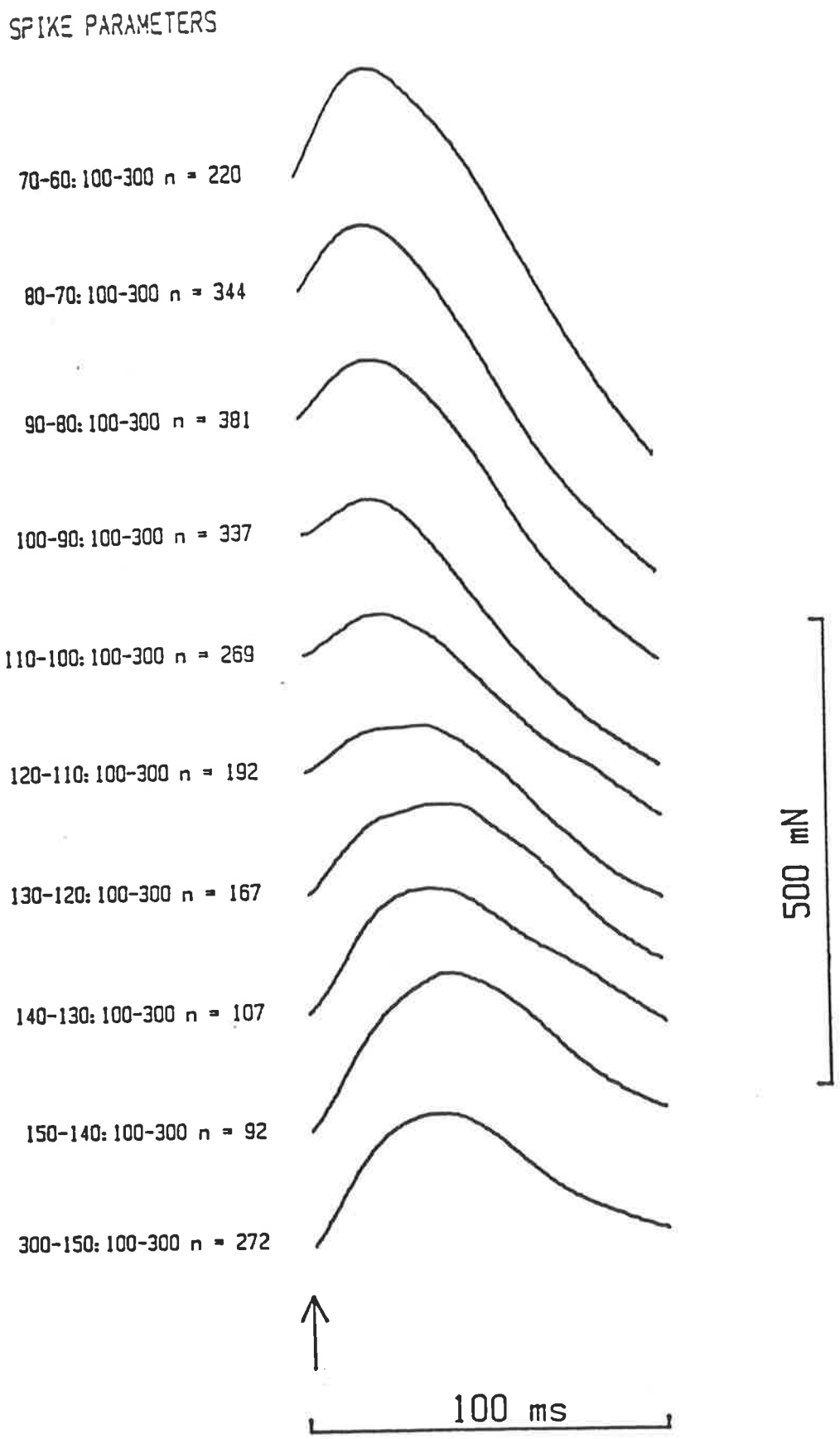
The averaged force records were plotted, and the twitch peak amplitude, time – to – peak tension (TTP), and half – relaxation time were measured from the plots. The averaged surface EMG and rectified surface EMG records were also plotted. Comparison of the rectified and unrectified EMG averages gives an estimate of the synchronous activity of other motor units in the muscle with respect to the triggering unit (Milner – Brown *et al.* 1973*a*; Milner – Brown *et al.*, 1975). The units used in this study did not show evidence of synchronous activity using the criteria of Milner – Brown *et al.* (1975). Long segments of continuous activity were used (9 – 15 min) in order to have a sufficient number of trigger spikes in each average because of the restrictive spike parameters used. The units chosen for analysis were those that exhibited little evidence of twitch fatigue over the analysis period, as judged by comparison of twitch amplitude estimated from successive 1 – minute epochs.

3.3 Results.

The effect of the preceding ISI on the twitch was examined in detail in five masseter motor units. In all cases the immediate firing history of the unit had a considerable effect on the twitch obtained by spike – triggered averaging. The pattern of changes in the twitches obtained when the spike parameters used for valid trigger spikes were changed was consistent for all units studied. The effect on the twitch of varying the time since the preceding spike for one representative unit is illustrated on a fast time – scale in Fig. 3.1. For the first average (top trace), the requirements for each valid trigger spike were that the previous spike had occurred between 60 and 70 ms before the spike in question, and that the next spike occurred between 100 and 300 ms after it. This will hereinafter be described in the form "spike parameters 70 – 60:100 – 300". For the subsequent averages in Fig. 3.1, the time between the preceding spike and the trigger spike was progressively increased in 10 ms increments, while the post – spike parameters were unchanged; that is, average no. 2 had spike parameters 80 – 70:100 – 300, and so on, ending with spike parameters 150 – 140:100 – 300. The effect of an interval

FIGURE 3.1

The effect of the pre – trigger ISI on the twitch obtained by STA. Each trace is the result of averaging the same 9 – min segment of data from a masseter single motor unit with the spike parameters indicated on each record. The time of occurrence of the trigger spike is shown by the arrow. The number of events averaged is shown beside each trace as n.


SPIKE PARAMETERS
70-60: 100-300 n = 220
80-70: 100-300 n = 344
90-80: 100-300 n = 381
100-90: 100-300 n = 337
110-100: 100-300 n = 269
120-110: 100-300 n = 192
130-120: 100-300 n = 167
140-130: 100-300 n = 107
150-140: 100-300 n = 92
300-150: 100-300 n = 272
500 mN
100 ms

greater than 150 ms since the previous spike was assessed with spike parameters 300 – 150:100 – 300.

As the minimum allowable interval since the unit had last fired was increased in 10 ms steps from 60 ms, the twitch initially decreased in amplitude until a minimum value was obtained with spike parameters 100 – 90:100 – 300. From this point onwards the twitch amplitude increased to a peak obtained at spike parameters 150 – 140:100 – 300. The amplitude of the twitch obtained when the previous action potential occurred between 300 and 150 ms before the trigger was similar to the peak twitch. The effect on the twitch time – to – peak (TTP) of increasing the time since the last spike was negligible between 60 and 100 ms. The TTP increased sharply from 21 ms to 32 ms at spike parameters 120 – 110:100 – 300, then remained fairly constant as the time since the last spike was steadily increased.

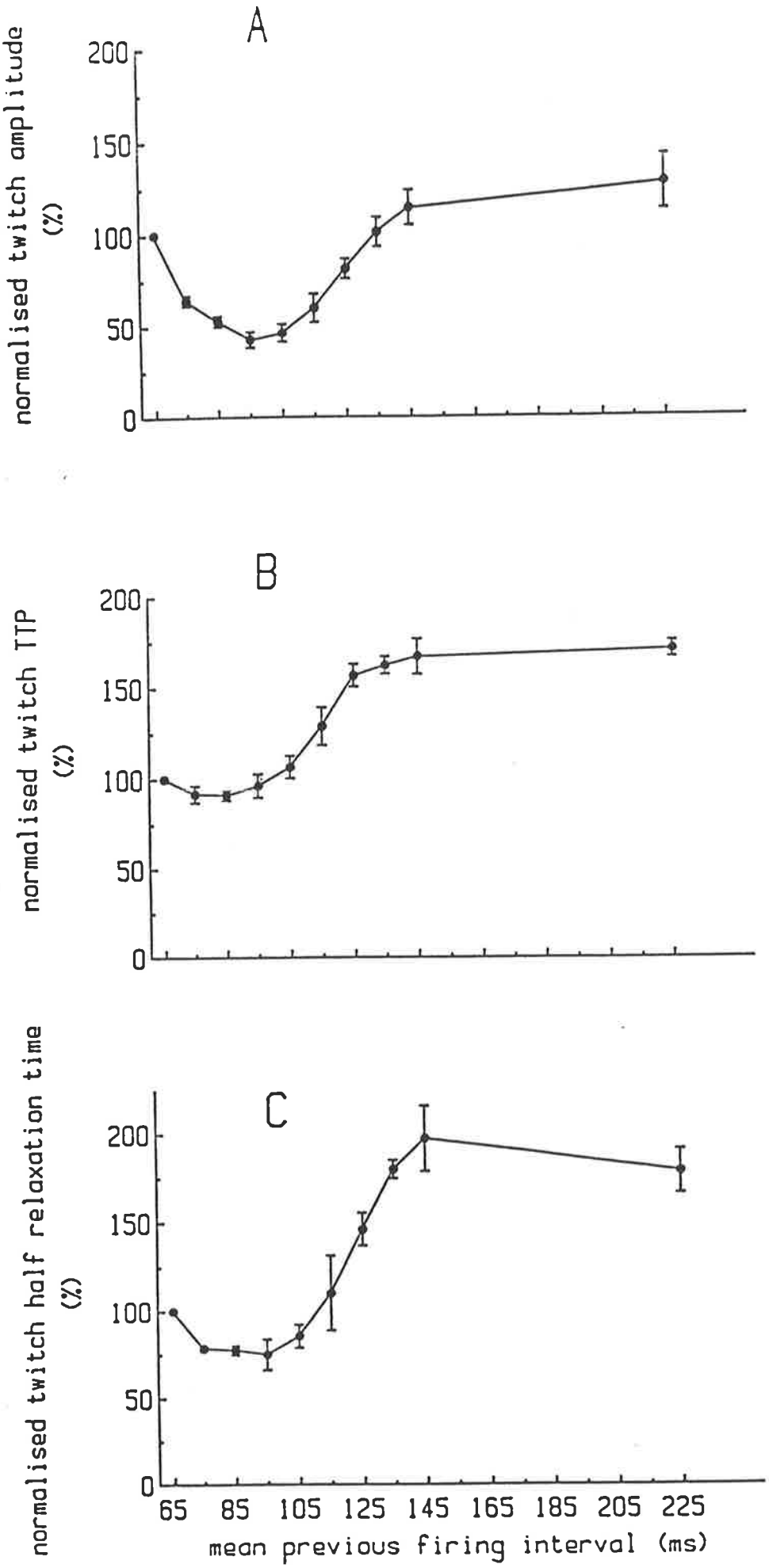
The normalised twitch data from all 5 units at various spike parameters are shown in Fig. 3.2. Twitch values were normalised as a percentage of the value obtained with spike parameters 70 – 60:100 – 300. These 5 units had a range of twitch TTP of 32 – 43 ms (mean 38 ms) with spike parameters 300 – 100:100 – 300. The mean twitch TTP measured with these spike parameters for the larger sample of masseter units reported in Chapter 5 was 34.8 $\pm$ 10.1 ms (mean $\pm$ SD, n = 57), and so this sample of masseter motor units was considered to be representative in terms of contractile speed. The pooled data show that, as the mean pre – trigger interval was increased, the twitch amplitude (Fig. 3.2*A*) initially decreased from the value at 65 ms, to reach a minimum value at 95 ms. In fact, the minimum twitch amplitude for all units was obtained with a mean pre – trigger interval of 95 ms. From this point, the twitch amplitude steadily increased as the pre – trigger interval increased. The values for intervals beyond 135 ms were not significantly different from each other (paired t – tests, $P > 0.05$).

The normalised twitch TTP (Fig. 3.2*B*) remained relatively stable as the time since the previous spike increased from 65 ms to about 105 ms, then increased sharply. The twitch TTP then remained relatively stable as the pre – trigger interval was increased beyond 130 ms. The normalised twitch TTP values obtained with mean previous firing intervals 125 ms or longer were not significantly different from each other (paired t – tests, $P > 0.05$).

The pattern of change in the twitch half – relaxation time (Fig. 3.2*C*) was similar to that of the twitch TTP. As the mean pre – trigger interval was increased from 65 ms, the half – relaxation time remained relatively constant, until a sharp

FIGURE 3.2

Pooled data from 5 units showing the effect of the pre – trigger ISI on the twitch obtained by STA. The twitch values were normalised as a percentage of the value obtained with spike parameters 70 – 60:100 – 300 for each unit, and the mean values (± SEM) for 5 units plotted for each set of spike parameters. The post – spike firing parameters were kept constant at 100 – 300, while the pre – spike firing parameters were varied systematically as in Fig. 3.1. The value for each set of spike parameters is plotted at the mid – point of the pre – spike firing parameters; i.e. the first point was obtained with spike parameters 70 – 60:100 – 300, and so the point is plotted at 65 ms on the horizontal axis. *A* shows the effect of previous firing history of the unit on normalised twitch amplitude, *B* on normalised twitch time – to – peak (TTP), and *C* on the normalised twitch half – relaxation time.


A
normalised twitch amplitude (%)
200
150
100
50
0
B
normalised twitch TTP (%)
200
150
100
50
0
C
normalised twitch half relaxation time (%)
200
150
100
50
0
65
85
105
125
145
165
185
205
225
mean previous firing interval (ms)

increase occurred with mean pre – trigger intervals between 115 and 135 ms. Increasing the pre – trigger interval beyond 135 ms did not significantly alter the twitch half – relaxation time values (paired t – tests, $P > 0.05$).

A clearer understanding of the effects of the pre – trigger interval on the twitches is gained by plotting the twitches on a slower time scale. Fig. 3.3 shows the same records as Fig. 3.1 on a slower time scale, with the averaged force shown for the period 200 ms before, and 300 ms after the trigger spike (the trigger position is indicated by the arrow below each trace). An unusual feature is the steep positive slope before the trigger in the top 3 traces. This was a characteristic pattern in the records from every unit tested with this series of spike parameters.

One possible explanation for this phenomenon is non – linear summation of force due to doublet firing of the unit in the period preceding the short interspike interval (ISI) of 60 – 80 ms. The force increment arising from doublet activation (less than 10 ms apart) is much greater than that following a single spike (Burke *et al.*, 1976). If an ISI of 60 – 80 ms is more likely to be preceded by a very short ISI (doublet firing), the effect on the force prior to the trigger could be similar to the steep increase in force seen in Fig. 3.3 when these spike parameters were used.

This possibility was excluded by using the spike selection facility of the averaging computer. For this purpose, the parameters for a valid spike for averaging were altered so that the pre – trigger interval parameters were kept constant, and the post – trigger parameters were varied systematically. By varying the intervals after the trigger spike, the force was averaged only with respect to single firings of the unit (i.e. not doublets), followed by another firing within a specifiable time window afterward. Fig. 3.4 shows averages obtained from the same epoch of data as that used for Figs. 3.1 & 3.3. The pre – trigger parameters constant, and the minimum post – trigger interval was progressively increased from 60 ms to 90 ms. The steep positive slope in the averaged force record following the trigger spike is still present in these records in which the smallest allowable pre – trigger interval was 100 ms, and the first post – trigger spike occurred between 60 – 90 ms after the trigger. The amplitude of this pre – twitch increase in force with interspike intervals of 60 – 90 ms is similar to that seen in Fig. 3.3. Therefore, it is not necessary to postulate doublet firing in the pre – trigger interval to explain the steep increase in force before the triggered twitch seen in the upper records of Fig. 3.3, and some other explanation must be sought.

FIGURE 3.3

The effect of the pre – trigger ISI on the averaged pre – and post – trigger force. Same data as Fig. 3.1, plotted on a longer time scale. The averaged force is shown for the period 200 ms before, and 300 ms after the trigger spike. Each trace is the result of averaging the same 9 – min segment of data with the spike parameters indicated at the left of each record. The time of occurrence of the trigger is indicated by the arrow below each trace. The time of occurrence of the closest pre – trigger spike allowed is indicated by the dot below each trace.

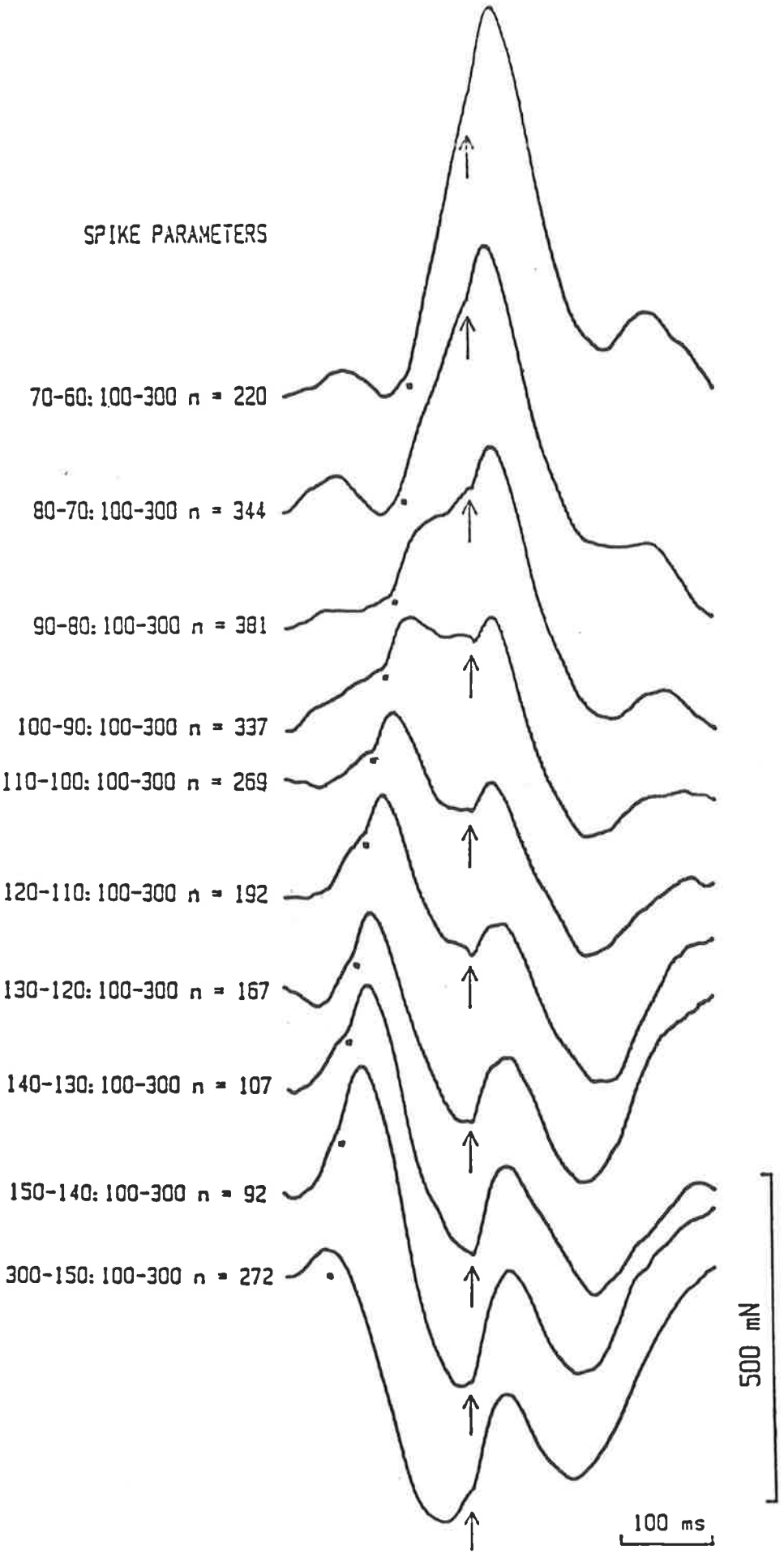
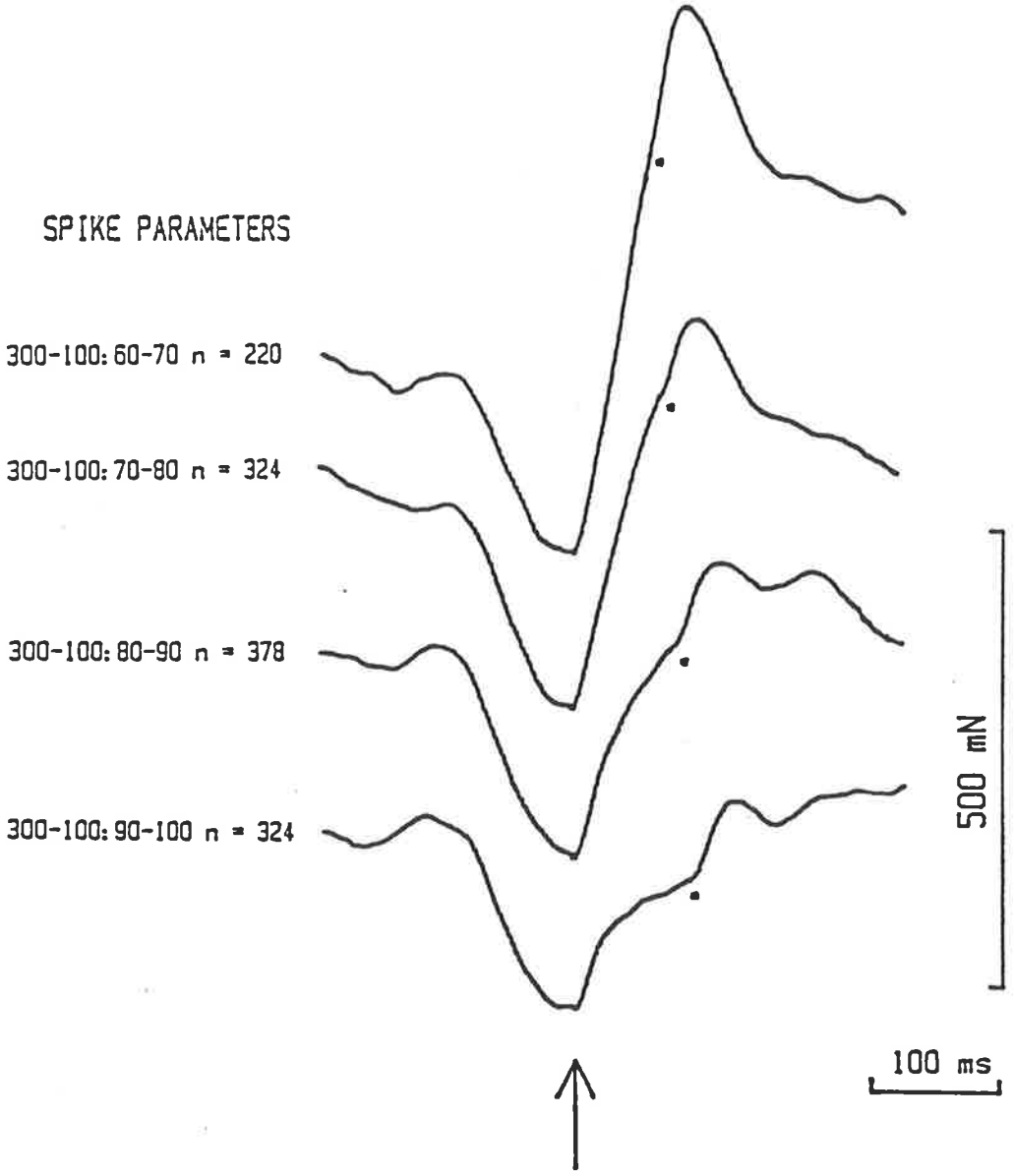

SPIKE PARAMETERS
70-60: 100-300 n = 220
80-70: 100-300 n = 344
90-80: 100-300 n = 381
100-90: 100-300 n = 337
110-100: 100-300 n = 269
120-110: 100-300 n = 192
130-120: 100-300 n = 167
140-130: 100-300 n = 107
150-140: 100-300 n = 92
300-150: 100-300 n = 272
500 mN
100 ms

FIGURE 3.4

The effect on the force averages of varying the time of occurrence of the first post – trigger spike. Each record is the result of averaging the same 9 – min segment of data as that used for Figs. 3.1 & 3.3, with the spike parameters indicated to the left of the trace. Note that the pre – trigger spike parameters are identical in each case, and the pre – trigger force changes are similar. The time of occurrence of the trigger is indicated by the arrows. The time of occurrence of the closest post – trigger spike allowed is indicated by the dot below each trace.


SPIKE PARAMETERS
300-100: 60-70 n = 220
300-100: 70-80 n = 324
300-100: 80-90 n = 378
300-100: 90-100 n = 324
500 mN
100 ms

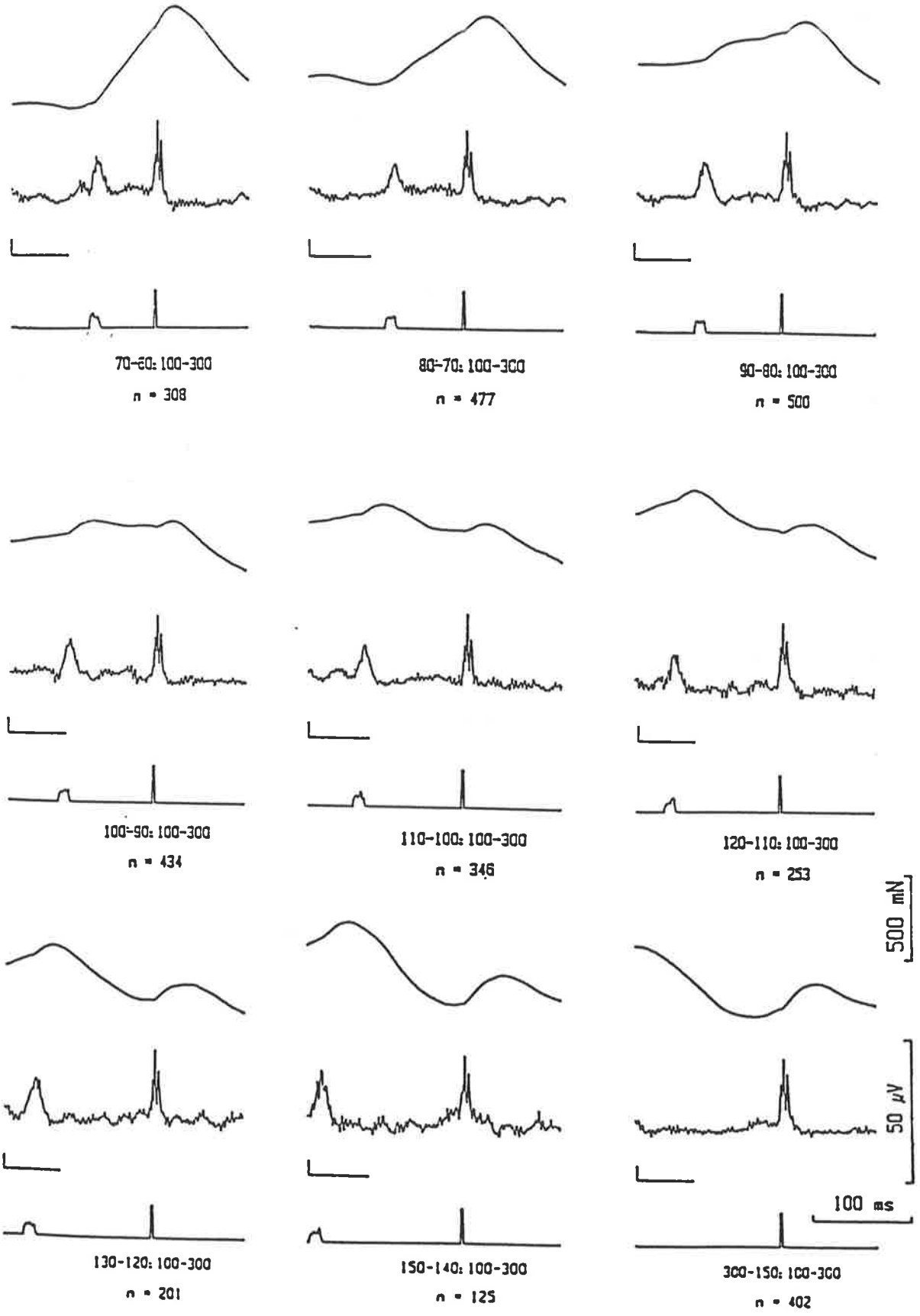
The pre – trigger parameters are identical in each case in Fig. 3.3, and the pre – trigger force changes are similar in each record. If the changes in post – trigger force were due to a change in the force output of the unit itself under the different conditions, it would be necessary to postulate that the force output of the unit due to the trigger spike was influenced by the time of occurrence of a future spike. This is not likely to be the case, and so the only other possible explanation is that the different post – trigger forces result from the activity of other units in the muscle.

This can be seen in the concurrently – averaged records of the rectified surface EMG. The averaged force and rectified EMG records for one unit obtained by averaging with various spike parameters are displayed on a slow time scale in Fig. 3.5. This is the same unit whose force averages are shown in Figs. 3.1, 3.3 & 3.4. The uppermost trace in each record is the force average. Below this is the averaged rectified EMG record, with the horizontal line immediately below this indicating ground potential. The lowermost trace in each record is an autocorrelogram of the spikes selected as valid triggers. The large narrow peak is from the trigger spikes, and the broad, smaller peak to the left shows the timing of the pre – trigger spikes. The spike parameters used for valid trigger spikes are shown immediately beneath each record, together with the number of triggers used for each average.

The rectified EMG records have a sharp peak associated with the contribution of the trigger unit to the surface EMG. There is also a broad secondary peak related to the pre – trigger spikes of this unit in the rectified EMG trace. The level of the rectified EMG between these two peaks and in the 100 ms following the trigger is a measure of the total activity of all other units in the muscle. In the record obtained with spike parameters 70 – 60:100 – 300 (top left) the rectified EMG level is noticeably higher in the period prior to the trigger spike than in the period following it. This shows that on average there was more activity in the other units in the muscle in the period just before the trigger spike than there was after it, with these spike parameters. The corresponding force trace shows a steep increase in force at the time of occurrence of the trigger spike, which is caused by this correlated activity of other units. As the time since the previous spike was increased, the difference between the pre – and post – trigger rectified EMG levels decreased: this is consistent with the progressive reduction of the slope of the averaged force in the pre – trigger interval at corresponding spike parameters. With pre – trigger firing intervals between 100 & 130 ms, the rectified EMG level is virtually identical before and after the trigger (Fig. 3.5),

FIGURE 3.5

The effect of the pre – trigger interval on the whole muscle's activity. The trigger unit was the one used for the force averages in Figs. 3.1, 3.3 & 3.4, and the surface EMG was recorded during the same recording period. The EMG data were averaged over a 13 – min segment in order to increase the number of counts for each average and thus improve the resolution of the rectified EMG averages. The same segment of data was averaged with different spike parameters to give the nine separate records. The spike parameters used for each average are shown immediately below each record. The value **n** refers to the number of events for the rectified EMG average. The top trace in each record is the averaged force. The middle trace is the average of the rectified EMG, with ground potential indicated by the horizontal line immediately below. The lowermost trace is the autocorrelogram of the unit showing the timing of other spikes in relation to the valid trigger spikes with each set of spike parameters. The large narrow peak in this trace is due to the trigger spike. The smaller broad peak to the left of this is due to the previous firings of the unit within the specified time window.


70-60: 100-300
n = 308
80-70: 100-300
n = 477
90-80: 100-300
n = 500
100-90: 100-300
n = 434
110-100: 100-300
n = 346
120-110: 100-300
n = 253
130-120: 100-300
n = 201
150-140: 100-300
n = 125
300-150: 100-300
n = 402
500 mN
50 µV
100 ms

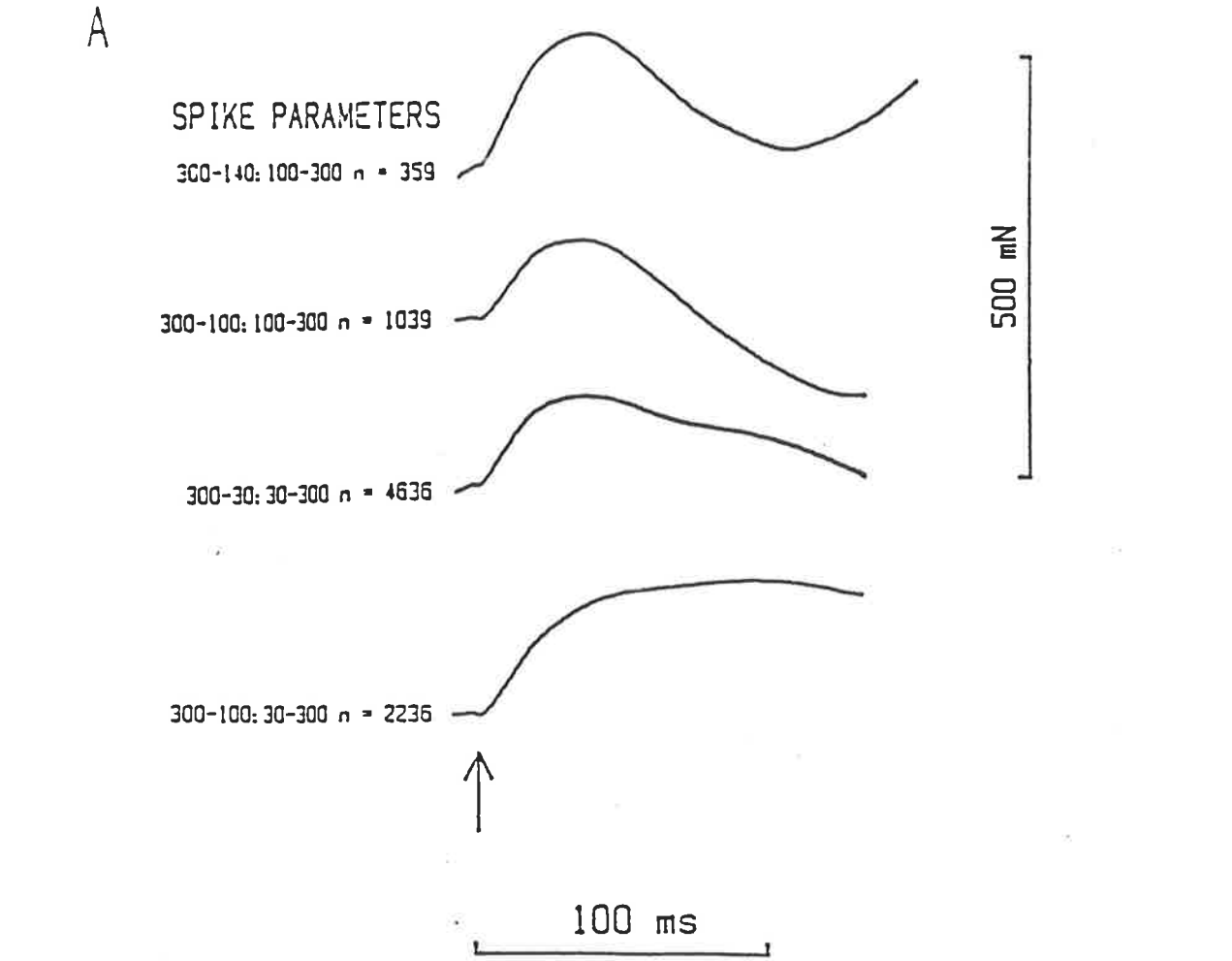
and the force trace at the corresponding spike parameters shows no evidence of increasing force in the period just prior to the trigger, but simply a progressive decrease in the averaged force associated with the previous spikes.

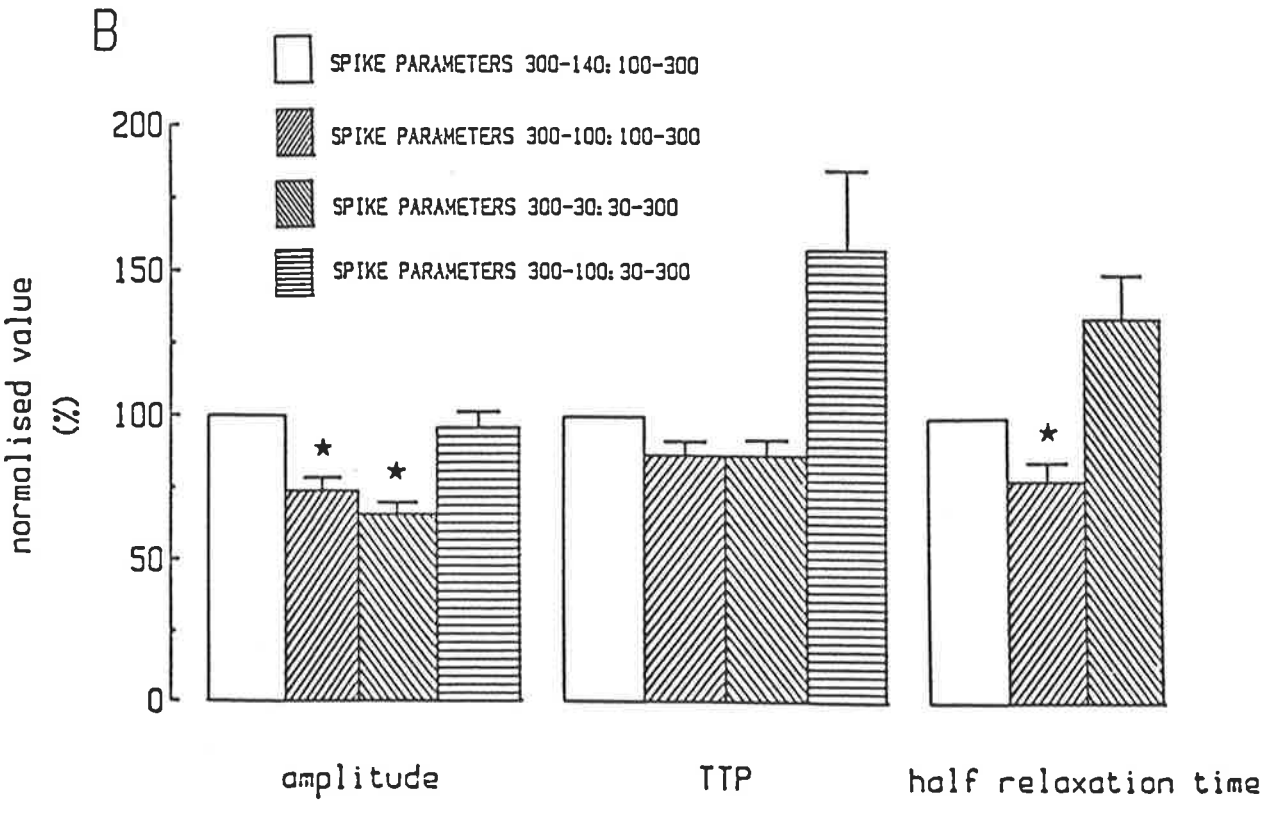
The two records at the lower right in Fig. 3.5 show that pre – trigger intervals greater than 140 ms resulted in a tendency for the averaged rectified EMG to increase in the period just before the trigger. Once again this correlated activity of other units is associated with an increase in the pre – trigger averaged force. Unlike the situation with short pre – trigger intervals, this minor degree of correlated activity did not seem to distort the twitch profile. The lowermost trace in Fig. 3.3 shows more clearly the force average resulting from using spike parameters 300 – 150:100 – 300 for this unit. Although the force was increasing just before the trigger with these spike parameters, the amplitude and TTP of the twitch was very similar to those in the two records immediately above (spike parameters 150 – 140:100 – 300 and 140 – 130:100 – 300) in which the force just before the trigger was either decreasing or slightly increasing. The pooled data showed that this was also the general pattern for all units with these spike parameters (Fig. 3.2).

The effect on the twitch of averaging the data with less – rigorous rate limitation, similar to that previously used in human STA, is shown for one representative unit in Fig. 3.6*A*. The uppermost trace shows the twitch obtained with spike parameters 300 – 140:100 – 300. As the spike parameters were changed so as to allow spikes with higher instantaneous firing rates to be accepted as triggers (Fig. 3.6*A*; middle traces), the main change in the twitch was a decrease in amplitude, and a large increase in the relaxation time with spike parameters 300 – 30:30 – 300. In contrast, the twitch obtained with spike parameters 300 – 100:30 – 300 (Fig. 3.6*A*; lower trace) had an increased amplitude, and a markedly increased TTP. The half – relaxation time was so prolonged that it could not be measured. For comparison of the group data, each twitch measurement for a unit was normalised with respect to the value obtained with spike parameters 300 – 140:100 – 300 (i.e. the least fused twitch). The mean $\pm$ SEM of these normalised values was calculated for the group at each set of spike parameters, and are displayed in the bar graphs in Fig. 3.6*B*. The twitch amplitude determined with spike parameters 300 – 100:100 – 300 and 300 – 30:30 – 300 was reduced to less than 75% of the value determined with spike parameters 300 – 140:100 – 300, and this was a significant difference in each case (paired t – tests, $P < 0.01$). Twitch TTP was not significantly different from the value at spike parameters 300 – 140:100 – 300 with any of the other 3 sets of spike parameters (paired t – tests, $P > 0.05$). Twitch

FIGURE 3.6

The effect of four different conditions of rate limitation on the twitches obtained by STA. The data from one unit is shown in *A*. Each trace is the result of averaging the same section of data with the spike parameters indicated to the left of each trace. The time of occurrence of the trigger spike is indicated by the arrow below the lowermost trace. The number of events averaged in each trace were: (from top to bottom) 359, 1039, 4636, 2236. *B*: Graph showing the pooled data for all 5 units with the 4 different rate limitation conditions. The twitch measurements for each unit were normalised with respect to the values obtained with spike parameters 300 – 140:100 – 300 (unshaded bars). The shaded bars show the mean $\pm$ SEM of these values for each set of spike parameters. Mean values significantly different (t – test, $P < 0.05$) from those obtained with spike parameters 300 – 140:100 – 300 are indicated by a star.


A
SPIKE PARAMETERS
300-140: 100-300 n = 359
300-100: 100-300 n = 1039
300-30: 30-300 n = 4636
300-100: 30-300 n = 2236
500 mN
100 ms


B
SPIKE PARAMETERS 300-140: 100-300
SPIKE PARAMETERS 300-100: 100-300
SPIKE PARAMETERS 300-30: 30-300
SPIKE PARAMETERS 300-100: 30-300
normalised value (%)
200
150
100
50
0
amplitude
TTP
half relaxation time

half – relaxation time was reduced significantly with spike parameters 300 – 100:100 – 300 (paired t – test, $P < 0.05$), but was not significantly different with spike parameters 300 – 30:30 – 300 (paired t – test, $P > 0.05$). No bar is shown at spike parameters 300 – 100:30 – 300, because with these parameters the relaxation phase of the twitch was so prolonged that the next spike occurred before the twitch declined to half amplitude, and so half – relaxation time could not be measured.

3.4 Discussion.

The results of this study show that the motor unit twitch obtained by STA in human masseter is greatly affected by the duration of the pre – and post – trigger ISI. As the minimum allowed pre – trigger interval was increased beyond 90 ms, the twitch amplitude progressively increased, and TTP and half – relaxation time became slower. This pattern is identical to that found by Calancie & Bawa (1986) when they stimulated cat hindlimb motor units at different rates, and results from the twitch becoming progressively less – fused as the pre – trigger firing interval was increased. In the present study, however, decreasing the minimum mean pre – trigger interval below 95 ms resulted in a paradoxical increase in twitch amplitude (Fig. 3.1). It is unlikely that non – linear summation of force is responsible, since very short ISI's were not present. It is expected that fusion would be greater with these spike parameters, and hence the twitch amplitude should continue to diminish at smaller pre – trigger intervals, as was found by Calancie & Bawa (1986). The reason for this deviation from the expected result in the present study appears to be the steeply – increasing slope of the averaged force preceding the trigger when the pre – trigger ISI was less than 90 ms (Fig. 3.3). Since the average force change associated with the firing of a unit was found to be affected substantially by the timing of a future spike (Fig. 3.4), the most likely explanation for this phenomenon is that it is due to correlated activity of other units in the muscle, rather than a difference in force output of the unit itself, when these short pre – trigger intervals were present. This explanation is further supported by the averaged rectified EMG records in Fig. 3.5. The motor unit twitch is therefore superimposed on a still – rising force, which markedly distorts the measurement of the amplitude and shape of the twitch. The slope of the pre – trigger increase in force became progressively greater as the pre – trigger interval was decreased below 90 ms (Fig. 3.3), which

probably accounts for the progressive increase in apparent twitch amplitude with these spike parameters (Figs. 3.2 & 3.3).

With pre – trigger intervals longer than 90 ms, the change in the twitch appearance with different firing rate parameters is consistent with a partially – fused twitch which became less – fused as the pre – trigger interval increased. There was a tendency for the averaged pre – trigger force to increase slightly with pre – trigger intervals longer than 140 ms. With these firing parameters, the firing rate of the trigger unit was actually increasing at the time of the trigger. This pattern of activation is accompanied by a correlated increase in activity of other units in the muscle (De Luca *et al.*, 1982*a*,*b*), as reflected by the increase in the rectified EMG level and force prior to the trigger with these spike parameters (Fig. 3.5). The twitch measurements did not appear to be distorted by this minor degree of correlated activity.

The demonstration of fusion of STA twitches in motor units under voluntary control in the present study is consistent with predictions of Calancie & Bawa (1986) based on stimulation studies in cat motor units, and also the computer simulation study of Andreassen & Bar – on (1983). The similarity of the twitches obtained with pre – trigger firing intervals greater than 130 ms in the present study suggests that the twitch is minimally fused at these slow instantaneous firing rates, which supports the prediction by Calancie & Bawa (1986) that the speed of contraction of motor units in human masseter muscle may be sufficiently fast to avoid fusion at these rates of activation.

In the study by Calancie & Bawa (1986), the degree of fusion at any particular rate of stimulation was greater in the slower – contracting motor units. The failure to confirm this pattern in the present study was probably due to the relatively uniform, fast contractile speed of the masseter motor units. It would be expected that for voluntary contractions involving slower – contracting motor units typical of other muscles, the effects of fusion at a mean firing rate of 10 Hz would be greater than those seen in the present study. However, direct demonstration of STA twitch fusion in voluntary contractions has been rare. Monster & Chan (1977) showed an example of twitch fusion in a motor unit of human extensor digitorum communis as the firing rate was increased from 8 to 16 Hz. In a study of the faster – contracting human masseter and temporalis motor units, varying the mean firing rate over a wide range (e.g. 8 – 15 Hz) had little effect on twitch amplitude or contraction time (Yemm, 1977*b*). Milner – Brown *et al.* (1973*b*) found no difference between the twitches obtained with mean firing

rates of 7 or 10 Hz in motor units of human FDI (range of twitch TTP 30 – 120 ms, mean about 55 ms). In that study it was also reported that motor unit twitches derived by STA were similar in amplitude and contractile speed to twitches obtained by intramuscular microstimulation at less than 2 Hz of (presumably) single units in the same region of the muscle in human FDI.

The reason for these discrepancies is not clear. Perhaps only the faster (larger – twitch) units were tested for fusion in FDI, and compared with intramuscular microstimulation. The rate control methods used in these studies to select the trigger spikes were less precise than those used in the present study. Yemm (1977*b*) relied solely on the ability of the subject to control the motor unit at the prescribed rate. Milner – Brown *et al.* (1973*b*) used a hardware rate – limiter off – line to restrict trigger spikes to an instantaneous firing rate of less than 7, or less than 10 Hz. The variability of motor unit discharge in voluntary contractions (Tokizane & Shimazu, 1964; Derfler & Goldberg, 1977) means that under these conditions, trigger spikes with a wide, and in some cases, common range of pre – trigger intervals were used for each average. This would tend to minimise the apparent differences between the twitches, and was not a factor with the precise rate control used in the present study.

In the second part of the present study the effect of these less – rigorous methods of trigger spike selection on the twitch was examined. The condition of no – rate – limitation other than the subject's ability to control the unit discharge at a mean rate of 10 Hz (e.g. Yemm, 1977*b*) was approximated using spike parameters 300 – 30:30 – 300. With these spike parameters, more than 95% of all spikes from the unit were accepted as triggers for the averager. Restriction of trigger spikes to instantaneous firing rates less than 10 Hz, and less than 7 Hz, (analagous to the method of rate limitation used by Milner – Brown *et al.*, 1973*b*) was achieved by averaging the same data with spike parameters 300 – 100:100 – 300, and 300 – 140:100 – 300 respectively. A simpler means of rate control (which also could easily be performed on – line using logic circuits) was imposed by considering only the pre – trigger interval for acceptance as a valid trigger. This does not seem to have been employed previously, although it would appear to be suitable and potentially useful for masseter motor unit twitches. Since masseter motor units rarely have ISI's less than 50 ms when firing with a mean rate of 10 Hz, and are fast twitch, it is reasonable to expect that the early time course of the twitch would be free from distortion due to a second firing of the unit. This form of rate control was simulated by averaging the data using spike parameters 300 – 100:30 – 300.

The effect of these rate limitation conditions on the twitch for all units is is summarised in Fig. 3.6*B*. The twitch values obtained with spike parameters 300 – 140:100 – 300 were used for comparison in each case, since it has been shown in the first part of the present study that the twitch is minimally fused with these spike parameters. The significant reduction in twitch amplitude of more than 25% with spike parameters 300 – 100:100 – 300 and 300 – 30:30 – 300 is indicative of greater fusion in the latter cases. Therefore, even with these less – precise rate limitation methods, there is still an observable difference in fusion of twitches obtained in the less than 7 Hz, and less than 10 Hz rate limitation conditions. With the subject attempting to control the mean firing rate of the unit at 10 Hz, there was not a great difference in the twitches obtained using rate limitation to restrict the instantaneous firing rate of the trigger spikes to below 10 Hz (spike parameters 300 – 100:100 – 300), and the no – rate – limitation condition (spike parameters 300 – 30:30 – 300). The increase in half – relaxation time in the latter condition, although not significant, was expected because of the acceptance of trigger spikes which were followed by another spike shortly afterward. The similarity of the twitches in these two conditions should not be interpreted as indicating a similar degree of fusion when spikes with pre – and post – trigger intervals below 100 ms are included in the average. On the contrary, this similarity is a fortuitous consequence of the steep force slope associated with intervals less than 90 ms (Figs. 3.3 & 3.4), which distorts the post – trigger force, and obscures twitch fusion.

The distortion of twitches obtained using spike parameters 300 – 30:100 – 300 (rate control on the pre – trigger interval only) was not expected. It was not due simply to the inclusion of trigger spikes with short post – trigger ISI's, because the early time – course of the twitch was affected in a period in which there were no additional spikes (determined from the autocorrelogram of motor unit firing; not shown). The explanation for this distortion of the twitch is that the short firing intervals are accompanied by correlated activity of other units which seriously distorts the force averages (compare with Fig. 3.4). This distortion is most severe in the apparent motor unit twitch when the short interval occurs after the trigger rather than before (compare the force averages in the period after the trigger in Fig. 3.4 with the top 3 traces in Fig. 3.3). Because of this, it is concluded that it is not valid to select trigger spikes for STA on the basis of the pre – trigger firing interval alone.

In summary, twitches obtained by STA are influenced by motor unit firing patterns, and care must be taken to interpret them correctly. It is likely that the

degree of fusion in muscles comprised of slower motor units would be greater than that demonstrated for masseter units firing at a similar rate. The STA technique appears to be suitable for determining the mechanical properties of human masseter motor units with minimal fusion, provided suitable rate limitation is imposed on the trigger spikes.

3.5 The Implications for the use of STA in the masseter for motor unit twitches.

The results in the previous section confirm that the pre – and post – trigger firing pattern of the motor unit can influence the twitch obtained with STA. In some cases the effect on the twitch is artifactual (e.g. the effect associated with pre – trigger intervals shorter than 90 ms), and in other cases the spike parameters determine the relative degree of twich fusion. It is therefore desirable to minimise this source of variability in order to increase the reliability of the twitch measurements, particularly when fatigue is to be assessed from changes in the twitch amplitude. The variability in the STA twitch associated with motor unit firing patterns was reduced by imposing constraints on the allowed pre – and post – trigger firing pattern for valid trigger spikes that were consistent for all units. The rationale for the choice of spike parameters for the fatigue study is given below.

As a general principle, it is desirable to average the force over the full time course of the twitch without distortion from other firings of the unit within the period immediately following the trigger spike. For this purpose it was necessary to impose limitations on the post – trigger firing interval for spikes selected as valid triggers. A lower limit of 100 ms was chosen because with the longest pre – trigger firing intervals (i.e. the least fused twitches) in this study, the averaged force had returned to near baseline level after about 100 ms (e.g. lower traces Fig. 3.1). An upper limit for the post – trigger firing interval was given an arbitrary value of 300 ms. Therefore the unit was required to fire again at least once in the 100 – 300 ms interval following a spike to satisfy the post – trigger selection criteria. This constraint ensured that the trigger spikes were occurring during a period of relatively steady firing (i.e., steady total force).

It is also desirable to select trigger spikes on the basis of the interval since the last firing of the unit in order to minimise the effects of fusion on the averaged twitch. The primary limitation here is that the unit must be discharging tonically so as to avoid the large force distortion that occurs with phasic activation due to

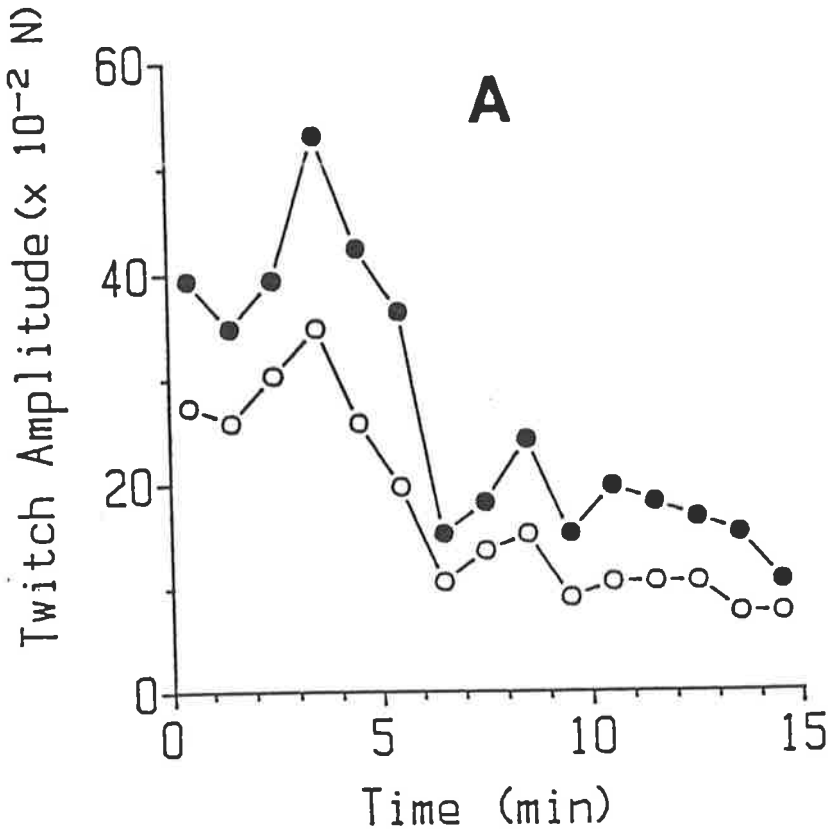
correlated activity of other units in the muscle (Yemm, 1977*b*). In order to avoid this type of distortion, valid trigger spikes were required to have a pre – trigger firing interval of less than 300 ms.

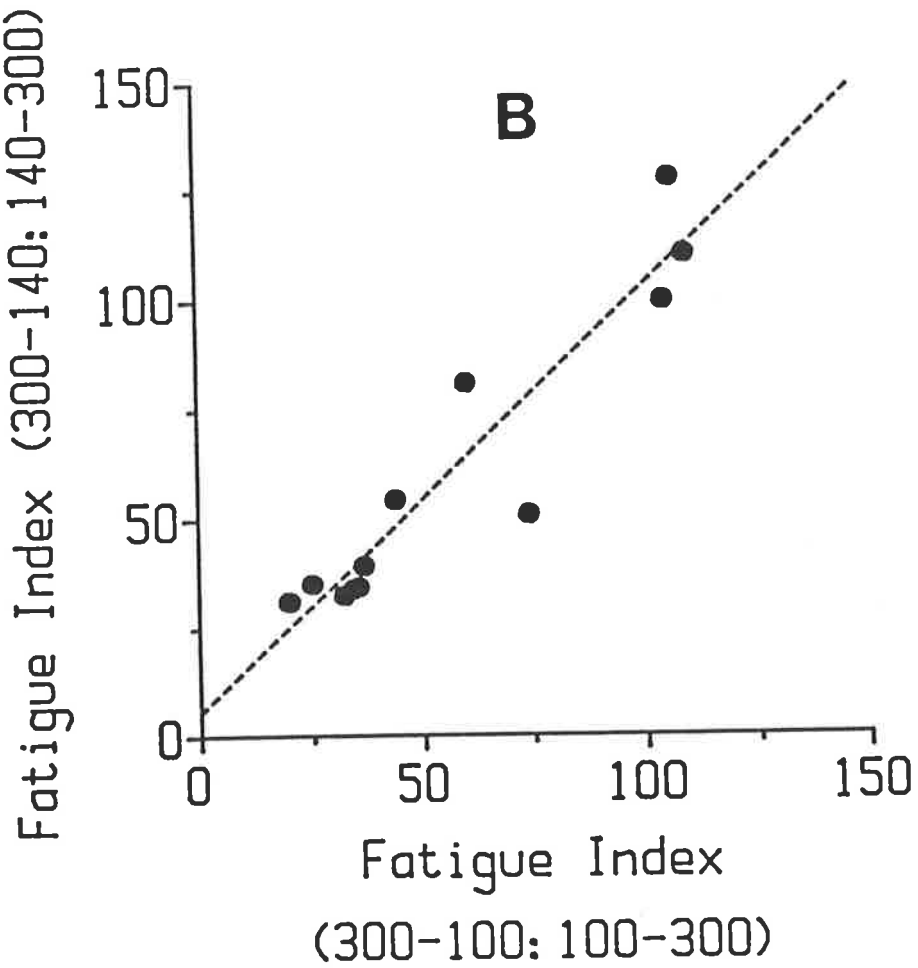
The lower threshold for tonic firing of masseter units was just below 10 Hz for most subjects, and subjects were instructed to control the trigger unit at this mean rate. In Section 3.3, it was found that masseter twitches could be obtained with minimal fusion using STA with spike parameters 300 – 140:100 – 300 when subjects attempted to control the unit at 10 Hz. However, the requirement for a tonic firing pattern meant that under these conditions the mean ISI was around 100 ms. That is, a large number of spikes were rejected as valid triggers when the spike parameters giving minimal fusion of twitches were used in the analysis. The desire to measure twitches with minimal fusion must be offset by the need for a finite number of triggers for a reliable average, which in turn determines the duration of the epoch needed to obtain a reliable twitch. The temporal resolution using spike parameters 300 – 140:100 – 300 was poor. A compromise was adopted for the fatigue tests (Chapter 5), which was to exclude spikes with a pre – trigger interval of less than 100 ms from triggering the average (i.e. spike parameters 300 – 100:100 – 300 were used). This eliminated from the averages those triggers associated with the steeply – increasing force ramps which distorted the twitch measurements (Fig. 3.3). It did mean that the resultant twitches were reduced approximately 25% in amplitude compared to the least – fused twitch (Fig. 3.6*B*). However, the number of valid triggers per epoch was dramatically increased, with the result that epochs as short as 1 minute could be used to detect significant changes in twitch amplitude.

The choice of spike parameters 300 – 100:100 – 300, rather than 300 – 140:100 – 300, did not distort the twitch fatigue indices. This was because the relative change in twitch amplitude with time was similar for the two sets of spike parameters. This is illustrated in Fig. 3.7. *A* shows the effect of averaging the same data with these two different sets of spike parameters for 1 – minute epochs during a 15 – minute contraction. The twitch obtained with spike parameters 300 – 140:100 – 300 was consistently larger, but the epoch – to – epoch values changed in parallel for each. The fatigue index (FI = 100 x [the twitch amplitude in the 15th minute divided by the twitch amplitude in the first minute]) was 26.4 with spike parameters 300 – 140:100 – 300, and 27.8 with spike parameters 300 – 100:100 – 300. In *B*, the FI was calculated in the same manner for 12 motor units using both sets of spike parameters. For each unit, the FI obtained with spike parameters 300 – 100:100 – 300 was plotted against the FI obtained using spike parameters

FIGURE 3.7

Effect of different spike parameters on twitch fatigue estimation. *A*: Each trace is the result of averaging the data from the same unit with different spike parameters. The filled circles represent the twitch amplitude calculated during 1 – minute epochs throughout the 15 – minute contraction, and obtained using spike parameters 300 – 140:100 – 300. The open circles represent the twitch amplitude obtained with spike parameters 300 – 100:100 – 300, for the same unit during the corresponding epochs. *B*: Pooled data from 12 units. For each unit, the FI obtained with spike parameters 300 – 100:100 – 300 was plotted against the FI obtained using spike parameters 300 – 140:140 – 300. The linear regression line – of – best – fit shown had a slope of 0.98 ($r = 0.94$).


A
Twitch Amplitude (x 10⁻² N)
60
40
20
0
0
5
10
15
Time (min)


B
Fatigue Index (300-140: 140-300)
150
100
50
0
0
50
100
150
Fatigue Index
(300-100: 100-300)

300 – 140:140 – 300. The slope of the linear regression line – of – best – fit was 0.98, and together with the small scatter ($r = 0.94$), this suggests that the two sets of spike parameters produced comparable fatigue indices.

CHAPTER 4.

IMPROVING THE RESOLUTION OF THE SPIKE-TRIGGERED AVERAGING TECHNIQUE.

4.1 Introduction.

The technique of ensemble averaging of event-related signals has been widely used in Neurophysiology to extract a small signal from a noisy background. Two methods are commonly used to improve the resolution of the average. The first is to filter the signal entering the averager so that unwanted frequencies do not contribute to the averaging procedure; this has the effect of increasing the signal-to-noise (S/N) ratio of the input signal to the averager. This technique is particularly useful when the bandwidth of the output signal is known in advance, although filtering may introduce unwanted (and often undetected) distortions of the output waveform. The second method is to increase the number of trials that are averaged. The latter approach, while often useful, is not always practical, since the event being studied may itself change with time (e.g., a fatiguing muscle twitch) or the number of trials may be limited for technical reasons (e.g., the deterioration of a cell membrane potential during an intracellular recording or the limited time for which an extracellularly recorded action potential can be "held" due to electrode movement). In any event, the improvement gained is only proportional to the square root of the number of trials, and is thus an approach of diminishing returns.

There are several situations in which the desired signal is superimposed on a background signal whose amplitude fluctuates widely. This problem is compounded if the bandwidth of the background waveform is similar to the bandwidth of the desired signal. This is the case when the technique of spike-triggered averaging (STA) is used to determine the twitch characteristics of single motor units in a contracting human muscle, as in the present investigations. In this paradigm, the subject must voluntarily contract the muscle in such a way that the motor unit under investigation fires at a steady rate of about 10 Hz with the help of visual and auditory feedback. However, when one examines the total muscle force signal that is to be input to the averager, two problems are immediately apparent. First, the force signal is usually offset considerably from ground potential, and second, the amplitude of the force signal fluctuates

considerably even when the unit is running at an acceptably steady frequency. The consequence is that the twitch signal itself will occupy only a small fraction of the input voltage range of the averager.

The conventional means of improving the S/N ratio in this situation is to high-pass filter the input signal to the averager using a filter with a time constant (τ) of 1.0 or 2.5 sec as suggested earlier (Milner-Brown *et al.*, 1973*a*). This has the desired effect of eliminating the offset potential, but the force fluctuations in which the twitch is "hidden" remain at an undesirably large amplitude to give reliable twitch values with the temporal resolution desired for the fatigue studies, particularly with the 2.5 sec time constant.

In the present experimental situation, it was desirable to have maximum temporal resolution of the twitch data in order to more accurately follow the time-course of fatigue. Therefore, the alternative of improving the reliability of the averages by increasing the number of trigger counts was not useable. The counts per unit time were limited to 10 per second because of the need to minimise twitch summation, and the only way to increase the number of counts in each average was to average over a longer epoch. Further improvements in the reliability of the twitches would have to come from improvements in the S/N ratio of the input signal to the averager. It was clear that the lower frequencies in the averaged twitch signal were close to the lower frequency limit of the filters; that is, the filters may have been distorting the actual twitch waveform, and further improvements with filtering were unlikely. The desired means of preparing the data for averaging should therefore preferably not involve filtering in order to avoid possible distortions of the twitch waveform, and should offer an advantage of a better S/N ratio than filtering.

The solution to this problem was a sample-and hold DC amplifier (SHA) which works in the following way. The input to the SHA is the raw force signal. Each action potential triggers the SHA to reset the force to near ground potential. The part of the signal that is of interest in the average occurs in the 100 ms or so after each action potential. Accordingly, if the input signal (force) is reset to ground potential by each action potential, the subsequent segment of the force signal containing the twitch can be led into the averager at a higher amplitude relative to the input voltage range of the averager.

4.2 Methods.

Apparatus and recording procedure.

These were identical to those described in Chapters 2 and 3. Subjects bit on the bite bars with their incisor teeth and controlled the firing rate of a selected motor unit at 10 Hz with the aid of feedback.

Circuit details and operation

A block diagram of the circuit is shown in Fig. 4.1*A*. The mode of operation of the SHA is shown in Fig. 4.1*B*. The slowly fluctuating input signal (middle trace in Fig. 4.1*B*) is offset some distance above ground potential. Each trigger pulse in the train (Fig. 4.1*B*: uppermost trace) forces the SHA output signal to near-ground potential (Fig. 4.1*B*: lowermost trace). From there, the output signal changes in parallel with the input signal until another trigger pulse resets it again to near-zero volts. This repositioned signal can then be amplified to a level appropriate to the input voltage range of the averager or it can be stored at high gain (i.e., with high S/N ratio) on FM tape for later off-line analysis.

To test the effectiveness of the SHA device I averaged the same experimental force data which had been treated in three different ways: (1) high-pass filtered with a time constant $\tau = 2.5$ sec, (2) high-pass filtered at $\tau = 1$ sec, and (3) with the SHA circuit. The gains of each of the three inputs to the averaging computer were adjusted so that the signal amplitudes did not exceed the ± 1 volt DC input range throughout the 20-second averaging period (left side of Fig. 4.2). Because of the slow fluctuations that remained in the force records after filtering, the maximal gain that could be used for these two inputs to the averager was 0.7V/N. By comparison, when the SHA was used to remove these force fluctuations, the maximal input gain possible was 1.5V/N.

The averaging computer selected valid trigger spikes for averaging using spike parameters 300-100:100-300. The same trigger spikes were used for the average of each of the three different force signals.

4.3 Results.

The result of averaging the same 20-second epoch of data under the three different conditions is shown on the right-hand side of Fig. 4.2. Each average (mean $\pm$ SEM) is plotted at the same gain. The peak amplitude of the twitch in

FIGURE 4.1

Block diagram of the SHA circuit and its operation. *A*. Block diagram of the circuit. The modus operandi of the circuit is to subtract the DC offset in the total force signal at the time of the trigger pulse from the total force signal with the summing amplifier. *B*. Operation of the SHA circuit. The analogue test signal (middle trace) has a DC offset of about 4.5 V. The DC component of this signal can be cancelled by the differential amplifier to near−zero volts on the occurrence of each of the trigger pulses in the uppermost trace: this produces the output shown in the lowermost trace. Note that the shape and amplitude of the output signal between successive pulses is unchanged from that of the input, but that the output is now centred around ground potential (0 VDC). This signal would now be led into an averager triggered by the pulses. The signals shown were recorded on a digital oscilloscope; hence the small steps in the three traces.

Sample-and-Hold Circuit

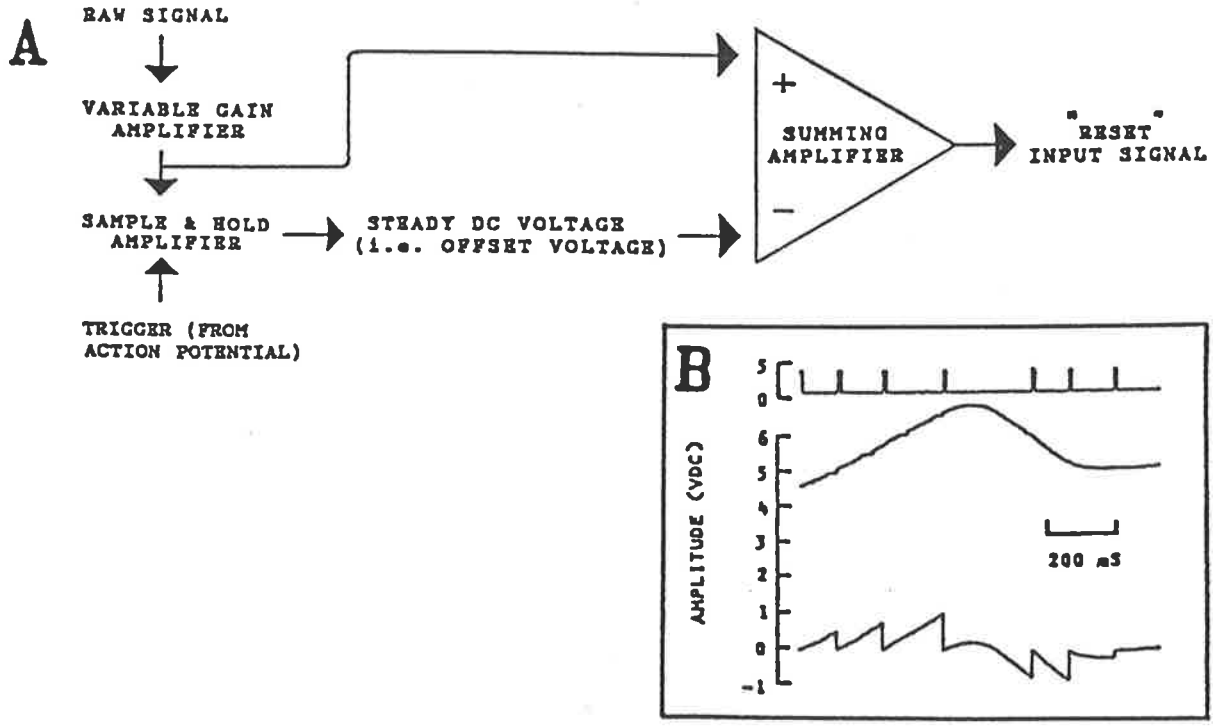
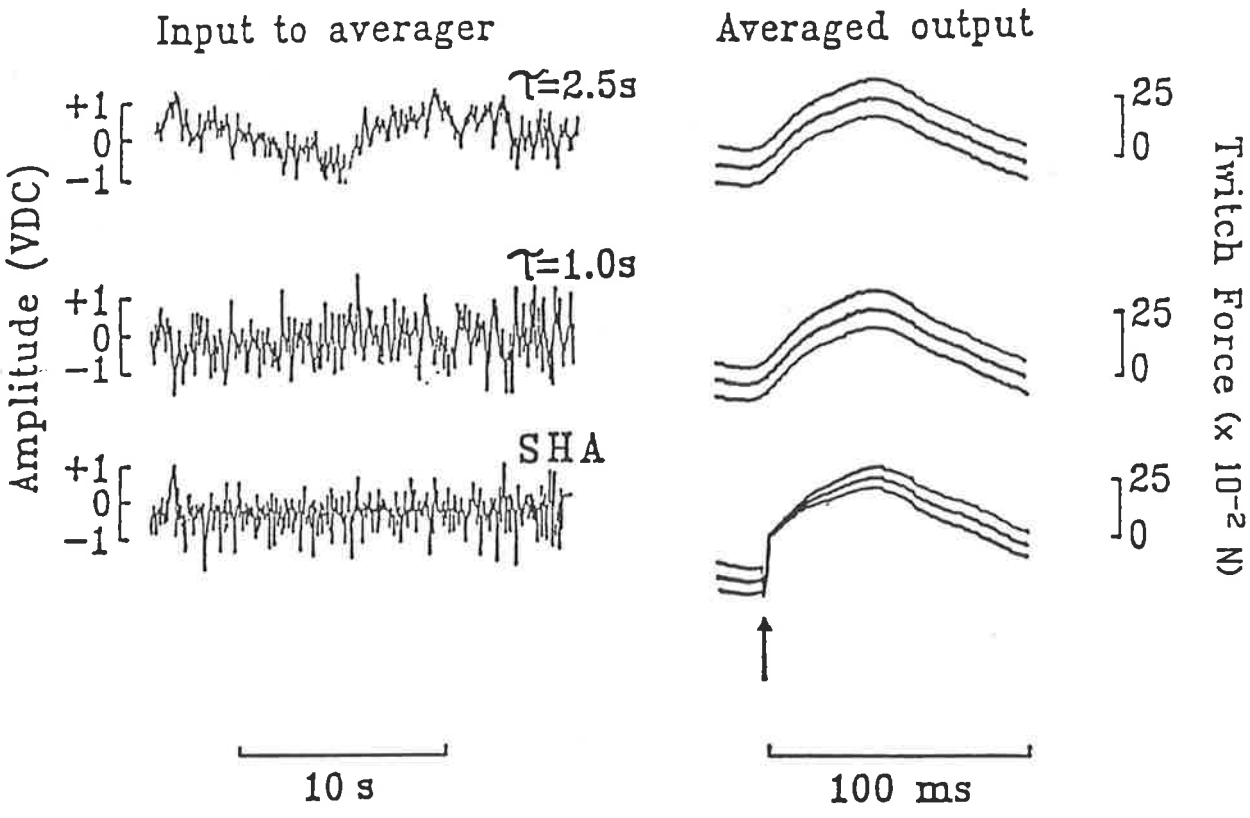


FIGURE 4.2

Comparison of the SHA approach with high – pass filtering when using spike – triggered averaging to measure single motor unit twitch force. The three traces on the left are the force input signals to the averaging computer; each one was adjusted to a ± 1 Volt range. The upper trace is the result of high – pass filtering the force signal with a time constant $\tau = 2.5$ sec, the middle trace is filtered with $\tau = 1$ sec, and the lower trace is the output of the differential amplifier. The traces on the right are the computer – derived means $\pm$ standard errors of twitch force corresponding to the three different input signals. The timing of the motor unit action potential that triggered the averager is indicated by the vertical arrow. Each average was obtained using the same 105 trigger spikes.


Input to averager
Averaged output
τ=2.5s
τ=1.0s
SHA
+1
0
−1
Amplitude (VDC)
25
0
Twitch Force (x 10⁻² N)
10 s
100 ms

both the filtered records and the SHA record was 250 mN in the example shown. However, at the peak of the twitch, the standard error of the mean for the filtered records was 92 mN, compared to 46 mN for the SHA.

4.4 Discussion.

The SHA circuit is designed to increase the precision of the STA technique when the signal to be averaged is offset from ground potential. It achieves this by resetting the input signal to the averaging computer or tape recorder to ground potential whenever a spike occurs. In this way, the signal that is to be averaged can occupy the whole of the input amplitude range of the averager or recorder; that is, the signal is played into the averager with a higher S/N ratio, which improves the precision of the average.

In the example given, the SHA enabled the input signal to the averager to be played in at approximately twice the gain than was possible with high – pass filtering. The standard error of the average obtained with the SHA input was then approximately half that obtained with the conventional filtering technique. The standard error gives an estimate of the reliability of the mean, and although it is directly proportional to the S/N ratio, it is inversely proportional to the square root of the number of counts in the average. In this example, therefore, one would need to use 400% more filtered trials than SHA trials to produce a similar level of reliability of estimation of twitch amplitude. This is a clear advantage in situations in which the signal that is to be measured is known to vary with time. For example, in the situation of measuring the change in twitch amplitude during a fatiguing contraction, the SHA enables successive averages to be made with a minimal number of spikes, that is, with an increased temporal resolution in comparison to the conventional filtering technique.

The SHA circuit also offers a major advantage in ease and accuracy of calibration when it is used for estimation of muscle twitches. The normal method for force calibration is to apply a series of static loads to the strain gauge and to record the steady DC output levels on FM tape or as a series of voltage levels that can be used to calibrate the amplitude of the output from an averager. When the high – pass filtering method is used, one cannot by definition use static loads for calibration signals, while with the SHA circuit, this is a straightforward procedure.

The general shape of the averaged twitch is very similar in both the filtered and SHA records. That is, the filtering procedure did not appear to distort the

waveform of the relatively fast – twitch masseter motor units. I have not attempted to compare the two techniques in averages of slower – twitch units in other muscles, in which the effect of filter – induced distortion is likely to be more prominent.

Finally, two potential traps for users of this device should be mentioned. The first is that the average of the force in the interval preceding the trigger is not reliable, as the input signal in this period is likely to be off – scale for the averager input in many trials. The sudden vertical deflection in the SHA average at the time of the trigger signal (Fig. 4.2) is due to the resetting of the input signal to zero volts by the differential amplifier at that instant. Second, it must be remembered that the input signal is reset to zero not only by the trigger spike but by all firings of the single unit under examination. Consequently, the use of this device must be restricted to situations in which a computer or some other device can be used to exclude trials in which trigger spikes occur within the post – trigger interval of interest. When averaging muscle twitches, this is necessary in any event to avoid summation.

In the experiments in the present series (Chapters 5 & 6), both the SHA and a high – pass filtered ($\tau = 1$ sec) force signal were averaged. The former gave the details of the shape and amplitude of the twitch with improved temporal resolution, while the latter gave an acceptable indication of the averaged events before and after the twitch. This device has been successfully used in this laboratory for STA of muscle force. It would also improve the resolution of STA in other applications, for example, in experiments in which nerve cell membrane potentials are averaged for the estimation of synaptic potentials.

CHAPTER 5.

FATIGUE OF SINGLE MOTOR UNITS IN HUMAN MASSETER

5.1 Introduction.

Despite the importance of the masseter as one of the major muscles of mastication, there is very little data on the physiological properties of its motor units. In fact, I have not found a single report in the literature in which mechanical properties of masseter motor units have been measured in animals using controlled electrical stimulation. An extensive classification of physiological properties of the masseter motor unit population in an experimental animal, similar to those carried out in a number of other muscles (Burke, 1981*a*), has not been performed. This is presumably due to the difficulty of access to the motor nerve, which precludes dissection and stimulation of individual motor nerve axons without damage to the muscle.

Even if extensive data on masseter motor units were available from a commonly-studied animal such as the cat, it is doubtful that it would be relevant to the situation in humans because of differences in fibre-type composition. The human masseter is composed predominantly of type I fibres (Eriksson & Thornell, 1983) whereas in the cat it is predominantly type II (Taylor *et al.*, 1973). In addition the human masseter appears generally to lack the type IIA fibres which correspond to the physiological type FR units (Eriksson & Thornell, 1983; Burke *et al.*, 1973). Other histochemical findings peculiar to the human masseter include the observations that the type I fibres usually have a larger diameter than the type II fibres (Ringquist, 1973*b*; Serratrice *et al.*, 1976; Vignon *et al.*, 1980; Eriksson & Thornell, 1983); both types I and II have a much smaller fibre diameter than the corresponding types in human limb muscles (Ringquist, 1971; Polgar *et al.*, 1973; Eriksson & Thornell, 1983); the mosaic pattern of fibre-type distribution normally found in limb muscles is absent (Eriksson & Thornell, 1983); and a high proportion of fibres show intermediate staining for myosin ATPase (Vignon *et al.*, 1980; Ringquist *et al.*, 1982; Eriksson & Thornell, 1983).

There is little physiological data available for human masseter motor units which might assist interpretation of the functional significance of these histological features. The two previous reports of the mechanical properties of masseter

motor units in humans using the spike – triggered averaging technique (STA) did not include fatigue testing (Yemm, 1977*b*; Goldberg & Derfler, 1977). The main goal of the present study was to measure the mechanical properties of the motor units in human masseter, including fatigue data for the units. This allows classification of the motor unit population of human masseter by the commonly – used classification scheme of Burke *et al.* (1973). It is widely believed that there is a good correlation between motor unit physiological type and the histochemical staining of the muscle fibres belonging to the unit (Burke, 1981*a*). Determination of the physiological properties of the units should then facilitate interpretation of the rather unique histochemical features of the human masseter. It should be emphasised that the masseter is particularly well – suited to an investigation of this kind. The masseter is a short, wide muscle which inserts into bone without a long elastic tendon. The connexion to the force transducer is via the teeth, which are effectively rigidly embedded in the bone (i.e. minimal soft tissue filtering), and can be reliably fixed in position so that the point and direction of application of force to the transducer remains constant. This eliminates many of the sources of distortion commonly encountered with the use of STA in other muscles.

A further goal of this study was to compare the fatiguability of human masseter units with that of motor units in other human muscles. It has been suggested that the masseter is more resistant to fatigue than non – masticatory muscles during certain submaximal and maximal fatigue tests (van Steenberghe *et al.*, 1978; Clark *et al.*, 1984; Clark & Carter, 1985). In the absence of motor unit fatigue data for the muscle it is difficult to speculate why this may be so.

5.2 Methods.

Apparatus and recording procedure.

All subjects were healthy adult volunteers aged 18 – 40 years with normal dentitions and no history of masticatory dysfunction. All subjects gave informed consent, and the experimental procedures were consistent with the recommendations of the Declaration of Helsinki for Human Experimentation.

The subjects bit on stainless – steel bite bars with their incisor teeth. The relationship of the jaws to the bars was kept constant by means of small, acrylic impressions of the subject's upper and lower incisal surfaces on the bars. The separation of the incisor teeth was fixed at 6 mm for the duration of the

contraction. Isometric biting force was measured by strain gauges mounted on the bars.

The force signals were recorded on 3 FM tape channels. In the first channel the total biting force was recorded in the bandwidth 0 – 1000 Hz. The other two channels contained the high – gain force records suitable for determining the twitch force of the single motor units. In one of these channels, the force was high – pass filtered ($\tau = 1$ sec), and amplified prior to recording. The signal in the remaining channel was the output of the sample – and – hold (SHA) amplifier (described in Chapter 4).

Motor unit activity was recorded with one or more bipolar electrodes inserted percutaneously into the right masseter muscle. The electrode consisted of 2 Teflon^R – insulated, stainless – steel wires (70 μm core diameter) threaded through the lumen of a 26 gauge disposable needle. After insertion, the needle was removed leaving the fine wires in place. In early experiments motor unit potentials were discriminated on – line using an amplitude – window discriminator. In later experiments the SPS 8701 was used for on – line motor unit identification. Each discriminated motor unit potential produced a TTL pulse which was led to a frequency meter. The mean motor unit firing rate (a weighted average of the previous 16 ISI's) was displayed as a horizontal line against a calibrated grid on an oscilloscope for subject feedback.

The surface electromyogram (EMG) of the right masseter muscle was recorded with bipolar Ag/AgCl electrodes. The skin surface was thoroughly prepared with alcohol and an abrasive paste. The gel – filled electrodes were placed about 2 cm apart near the centre of the muscle and aligned to the long axis of the muscle fibres. The muscle EMG signals were amplified (1000x) and recorded on analog FM tape (bandwidth 0 – 2500 Hz).

Protocol.

The subject was seated comfortably with the incisor teeth on the bite bars so that he/she could observe an oscilloscope screen on which was initially displayed the biting force and a ramp – shaped target line. Several slow force – ramps were tracked by the subject in order to determine the activation force threshold of the unit. After the ramps, the subject was allowed to rest for 1 minute before the start of the fatigue test. The visual feedback was then switched to display the mean firing rate of the selected unit, and the subject was instructed to bite isometrically on the bars with the force necessary to keep the unit firing

continuously at a mean rate of 10 Hz, for 15 minutes. The 10 Hz rate was necessary to minimise summation of the twitches.

Most subjects participated in a number of separate experimental sessions on different days. On some occasions the subject was allowed to rest for 20 – 45 minutes following a test run, after which the fine wire electrode was repositioned by a gentle pull to select a different unit, and the fatigue test was performed on the new unit. The unit chosen for testing during a second trial was required to have a higher activation force threshold than the unit in the first trial, and was therefore unlikely to have been active during the initial contraction.

Analysis.

Single unit spike trains.

The intramuscular EMG records were analysed off – line to generate trigger pulses for the averager. Single motor units were discriminated using either an amplitude – window discriminator or the SPS 8701.

Spike – triggered Averaging.

Test for synchronization.

The degree of synchronization of firing of the trigger unit with other units in the muscle will influence the accuracy of the STA technique. For all fatigue – tested units, a gross estimate of the tendency for synchronous discharge with other active units was obtained by STA of both the unrectified and rectified surface EMG signals (Chapter 2). Units were tested at the beginning and end of the contraction, and those exhibiting evidence of synchronous activity according to the criteria of Milner – Brown *et al.* (1975) were excluded from further consideration. Cross – correlation of motor unit firing times revealed weak synchronization in masseter motor units, and no evidence for time – dependent changes in the strength of synchrony (Chapter 2). In Section 2.5 it was argued that the observed pattern of masseter unit synchronization would result in only minor errors in twitches obtained by STA.

Single motor unit twitches.

A computer (PDP 11/73; sampling rate 1000 Hz per channel) was used to average the twitch data. Both high – gain force records were averaged. The high – pass filtered force record was used primarily as a guide to the averaged force in the pre – trigger interval, and as a check to ensure that the post – trigger

force average was similar to that obtained by averaging the SHA force. This record was filtered in the bandwidth 1 – 500 Hz prior to averaging. Measurement of twitch parameters such as twitch amplitude and time – to – peak (TTP) were made from the SHA average, except in some early experiments where the SHA force was not available. The SHA force record was low – pass filtered prior to averaging (Bandwidth 0 – 500 Hz). A special – purpose averaging program was used in which a unit potential was accepted as a valid trigger for the averaging procedure on the basis of its pre – and post – trigger firing interval. The spike parameters used for all averages were 300 – 100:100 – 300 (see Chapter 3). These constraints were necessary to minimise and control distortion of the STA twitch due to the firing pattern of the unit, as has been described in Chapter 3.

For each unit, the force record was averaged using both 1 and 3 minute epochs throughout the 15 minute contraction. The stringent firing pattern constraints resulted in less than 20% of all action potentials of the single unit being accepted as valid triggers; about 100 triggers were used to produce the averaged twitch records in most 1 minute epochs. The averaged twitches (mean $\pm$ SE) for each epoch were plotted. The peak twitch amplitude $\pm$ SE, and the twitch time – to – peak (TTP) were measured from the plots. Three indices of twitch fatigue were calculated:

i) FI_6 = 100 x (twitch amplitude in the 6th minute / twitch amplitude in the 1st minute). This index facilitated comparison with other human motor unit fatigue studies.

ii) FI_{15a} = 100 x (twitch amplitude in the 15th minute / twitch amplitude in the 1st minute). The 15 minute test was used in an attempt to subdivide the population of relatively fatigue – resistant units.

iii) FI_{15b} = 100 x (twitch amplitude in the 5th 3 min epoch (i.e. mins 13 – 15) / twitch amplitude in the 1st 3 min epoch). Three – minute epochs reduced the variability of individual twitch measurements giving a more reliable fatigue index, but at the cost of poorer temporal resolution.

Total biting force.

The activation threshold force of the single motor units was determined off – line from the force ramps. The subject's maximal incisal biting force (MBF) was recorded on a previous occasion, and was used to assess the relative strength of the contraction. The total biting force was plotted against time for the 15 minutes.

5.3 Results.

Twitch data was obtained for 57 masseter units from 14 subjects. The relationship between the motor unit activation force threshold and twitch tension for these units is shown in Fig. 5.1*A*. There was a significant positive correlation between these two variables ($r = 0.72$; $P < 0.001$). Fig. 5.1*B* shows the relationship between activation threshold force and twitch time-to-peak (TTP) for the same units. There was no significant correlation between them ($r = 0.07$; $P > 0.05$).

Fig 5.2*A* is a histogram of the initial twitch tension of the units. In each histogram in Fig. 5.2 the shaded bars represent the values from units that were fatigue tested (see below). The distribution of motor unit twitch tension was highly skewed towards small twitch tensions. The twitch TTP was distributed relatively symmetrically about a mean value of 34.8 ms ($\pm$ 10.1 SD), although there were several units with TTP > 60 ms (Fig. 5.2*B*). Fig. 5.2*C* is a histogram of the unit's activation force thresholds, which shows that the majority of the units were recruited below a total biting force of 15 N. The maximum incising force of the subjects ranged from 150-215 N, so most of these units were recruited below 10% MBF.

Not all units from which twitches were obtained and included above were fatigue-tested. For example, some were recorded (at an opening of 6 mm, being the initial position) in the length-change experiments (Chapter 7). Others could not be followed continuously for 6 minutes due to a change in action potential waveform which precluded accurate discrimination, so that the fatigue trial was aborted. The introduction of the SPS 8701 in later experiments alleviated this problem. Fatigue data was obtained for 37 units from 8 subjects. The FI_6 was measured for all units, and the FI_{15} was obtained for 32. The initial twitch amplitude (measured in the first minute of activity) of the 37 fatigue-tested units ranged from 20-780 mN. These units were recruited over a range of biting forces of 1-57 N, which represented a range of 0.7-26.6% MBF. Of the fatigue-tested units, 75% had activation thresholds below 10% MBF. Fig 5.3*A* shows the relationship between the fatigue-tested units' activation force threshold and their initial twitch tensions measured in the first minute of the contraction. These two variables were significantly correlated ($r = 0.69$, $P < 0.001$). The twitch TTP of the 37 units ranged from 20-72 ms (34.7 $\pm$ 10.4 ms, mean $\pm$ SD). The relationship between activation threshold and twitch TTP for these units is shown in Fig 5.3*B*; there was no significant correlation between them ($r = 0.10$, $P > 0.05$).

FIGURE 5.1

The relationship between motor unit activation force threshold and twitch amplitude (*A*) and TTP (*B*) for 57 masseter motor units. The slope of the linear regression line shown in A was significantly different from zero ($r = 0.72$; $P < 0.001$).

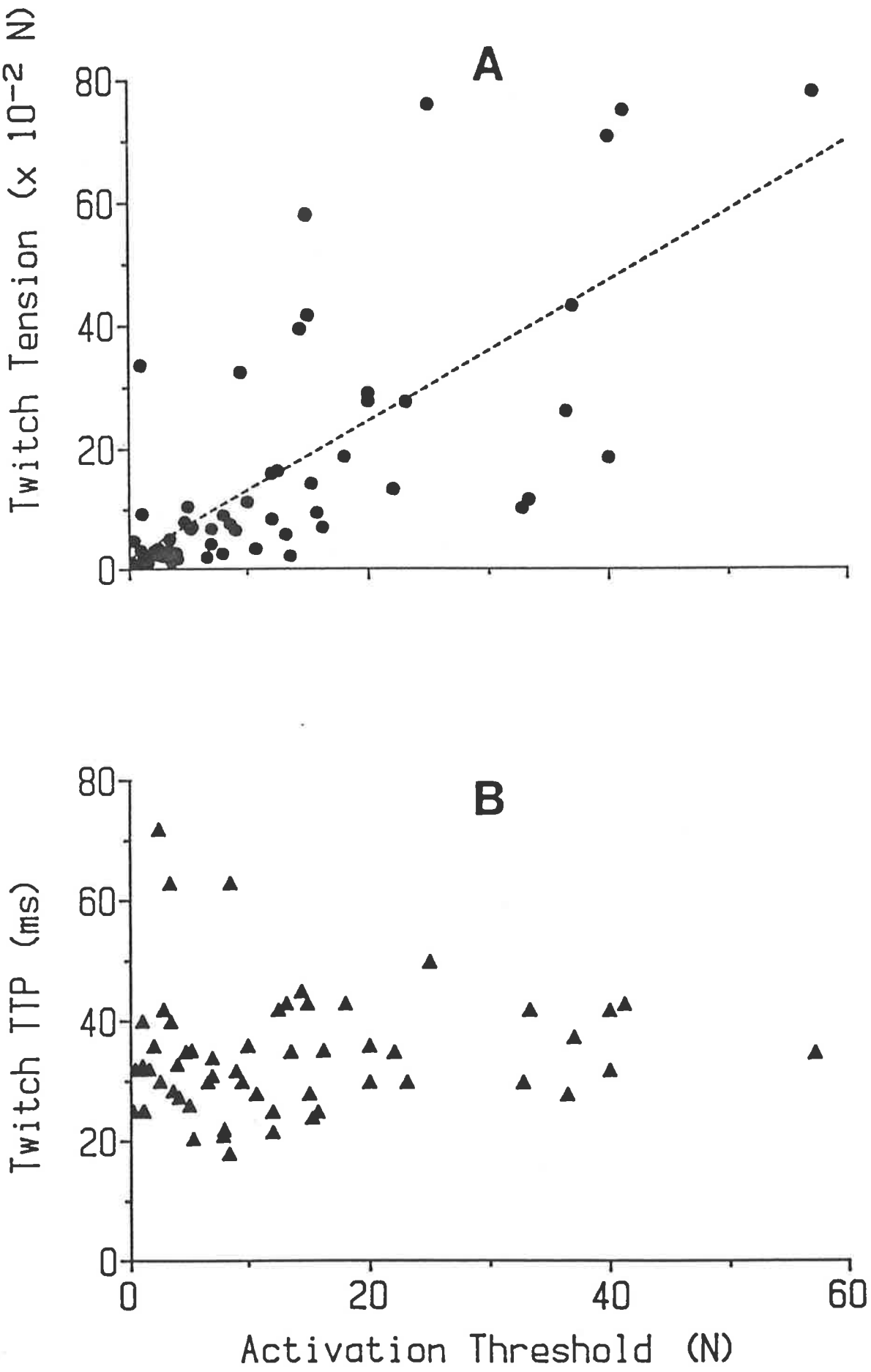

A
Twitch Tension (x 10⁻² N)
80
60
40
20
0
B
Twitch TTP (ms)
80
60
40
20
0
0
20
40
60
Activation Threshold (N)

FIGURE 5.2

The distribution of twitch tension (*A*), TTP (*B*), and activation force threshold (*C*) among 57 masseter motor units. The units from which a fatigue index was calculated are represented by shaded bars. Units that were not fatigue – tested are represented by the open bars.

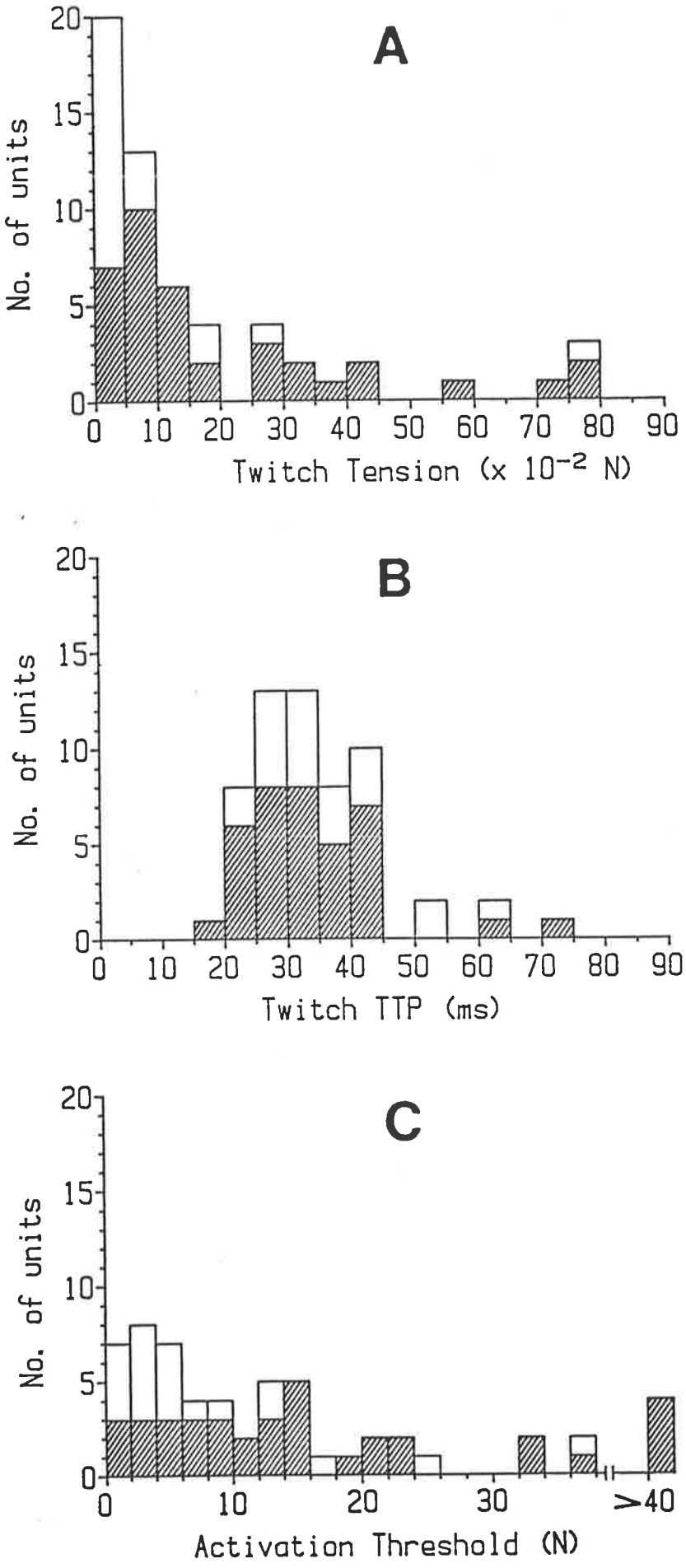
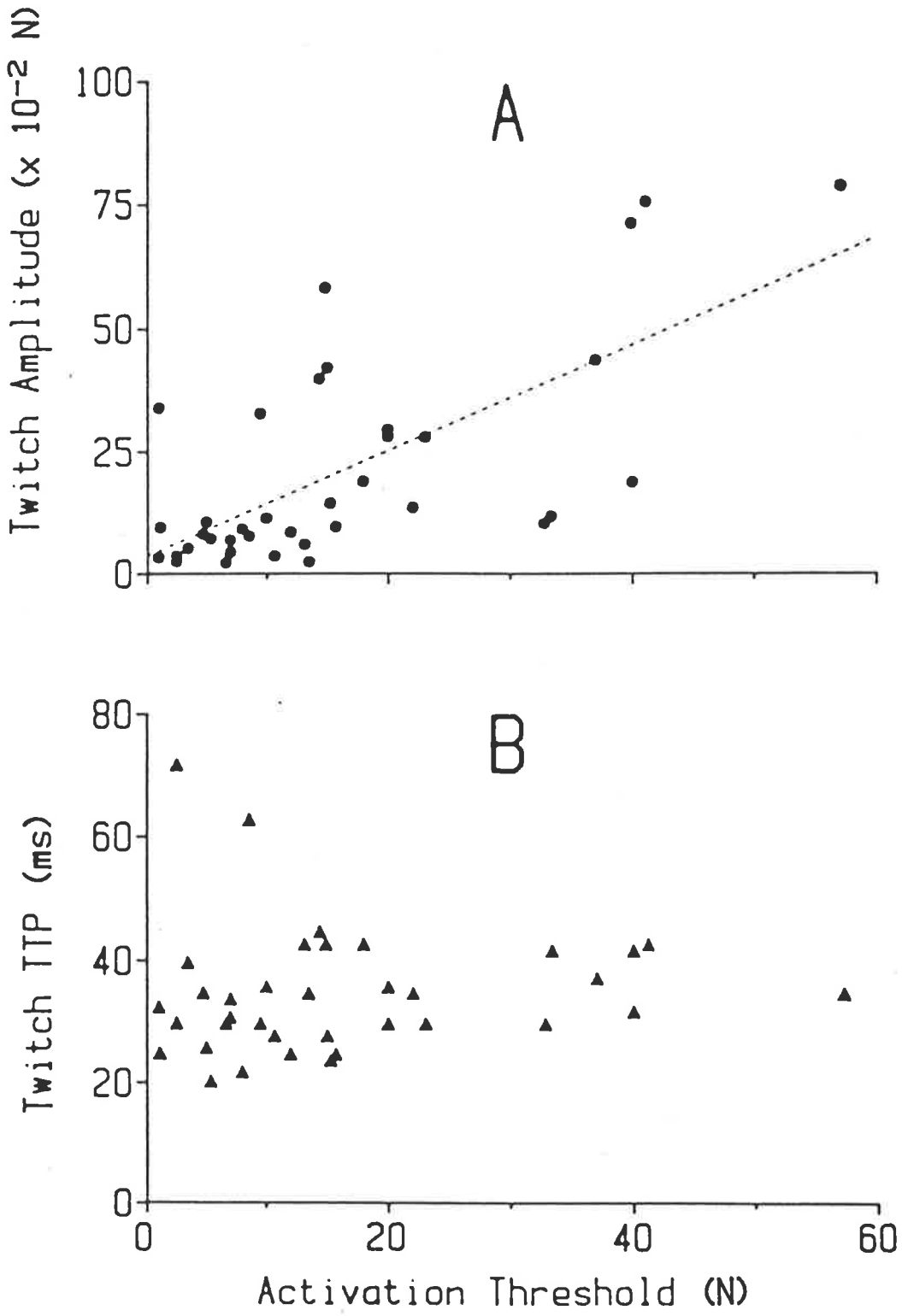

A
No. of units
20
15
10
5
0
0 10 20 30 40 50 60 70 80 90
Twitch Tension (x 10⁻² N)
B
No. of units
20
15
10
5
0
0 10 20 30 40 50 60 70 80 90
Twitch TTP (ms)
C
No. of units
20
15
10
5
0
0 10 20 30 >40
Activation Threshold (N)

FIGURE 5.3

The relationship between motor unit activation force threshold and twitch amplitude (*A*) and TTP (*B*) for 37 fatigue – tested masseter units. The slope of the linear regression line shown in *A* was significantly different from zero ($r = 0.69$; $P < 0.001$).


A
B
Twitch Amplitude (x 10⁻² N)
Twitch TTP (ms)
Activation Threshold (N)
100
75
50
25
0
80
60
40
20
0
20
40
60

The distribution of twitch TTP was essentially unimodal, although there were 2 units with TTP slower than 60 ms that were distinct from the main group.

The effect on the twitch of 15 minutes of continuous activation at 10 Hz is shown for 3 units in Fig 5.4*A* – *C*. The twitches measured in the first and 15th minute are shown on the left. Note that all 3 units had a similar initial twitch amplitude. In *A*, the twitch increased over the 15 minute period, whereas in both *B* and *C* the twitch amplitude decreased during the test. On the right side of Fig. 5.4, each unit's averaged twitch amplitude from each 1 minute epoch during the contraction are plotted. In unit *A*, the twitch amplitude increased over the first 4 minutes, and then remained relatively stable beyond 5 minutes. In unit *B*, the tendency was for a slow, progressive decline in twitch amplitude over the 15 minutes, with some fluctuation from epoch to epoch. Values for the last 5 minutes were similar. The majority of the units exhibited this pattern of gradual change in twitch amplitude with time. Twitch amplitude values from successive epochs in most units showed less fluctuation near the end of the 15 minute test.

Unit *C* differed markedly from *A* and *B* in that the twitch fatigued rapidly to 29% of its initial amplitude by the third minute, and remained relatively constant for the remainder of the test without periods of recovery. This was the typical pattern for the rapidly – fatiguing units ($FI_6 < 25$).

The twitch amplitude increased over the first few minutes of the contraction in many units. This is particularly evident in Fig 5.4*A*, where the unit's twitch amplitude increased by more than 100% between the 1st and 4th minute. Of the 32 units tested for 15 minutes, the maximum twitch amplitude was obtained during the initial minute of the contraction in only 9 (28%). The twitch was frequently maximal in the 2nd (16%), 3rd (16%) or 4th (19%) minute. Four units (12%) had their maximal twitch amplitude beyond 10 mins of activity.

The relationships between the various indices of fatigue and twitch amplitude and TTP are shown in Fig 5.5. The relationship between initial twitch amplitude and FI_6 for 37 units is shown in Fig 5.5*A*, with the relationship between twitch TTP and FI_6 shown in Fig 5.5*B*. Despite the wide range of FI_6 values and twitch amplitudes in the motor unit sample, there was no significant correlation between them ($r = -0.05$, $P > 0.05$). There was also no significant correlation between twitch TTP and FI_6 ($r = 0.15$, $P > 0.05$).

Extending the fatigue test to 15 minutes did not reveal a significant correlation ($P < 0.05$) between FI and either of these twitch parameters. In Fig 5.5*C* & *D*, the initial twitch amplitude and TTP are plotted against FI_{15a} for 32 units: the

FIGURE 5.4

Twitch fatigue in 3 masseter motor units. The averaged motor unit twitches calculated during the 1st and 15th minute of the contraction for 3 different motor units are shown on the left in $A-C$. The uppermost trace in each pair is the twitch calculated during the 1st minute, and the time of occurrence of the trigger is indicated by the arrow. The vertical calibration bar is 200 mN, and the horizontal bar is 50 ms. Immediately to the right of each pair of twitches, the twitch amplitude measured in each 1 min epoch for the corresponding unit is plotted over the 15 minutes. Note the different vertical scale in each. The error bars represent $\pm$ 1 SE of the mean twitch amplitude in each epoch.

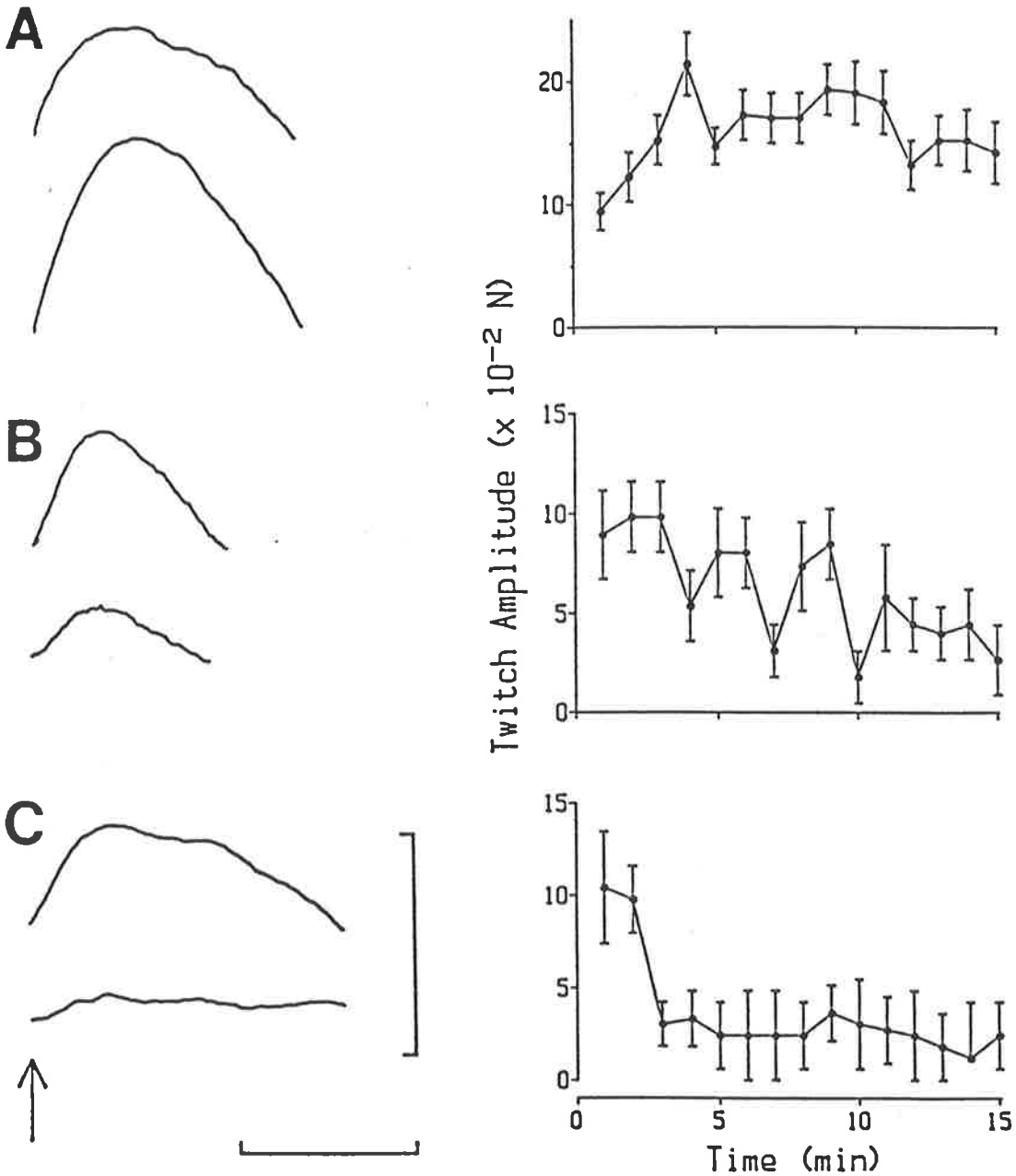
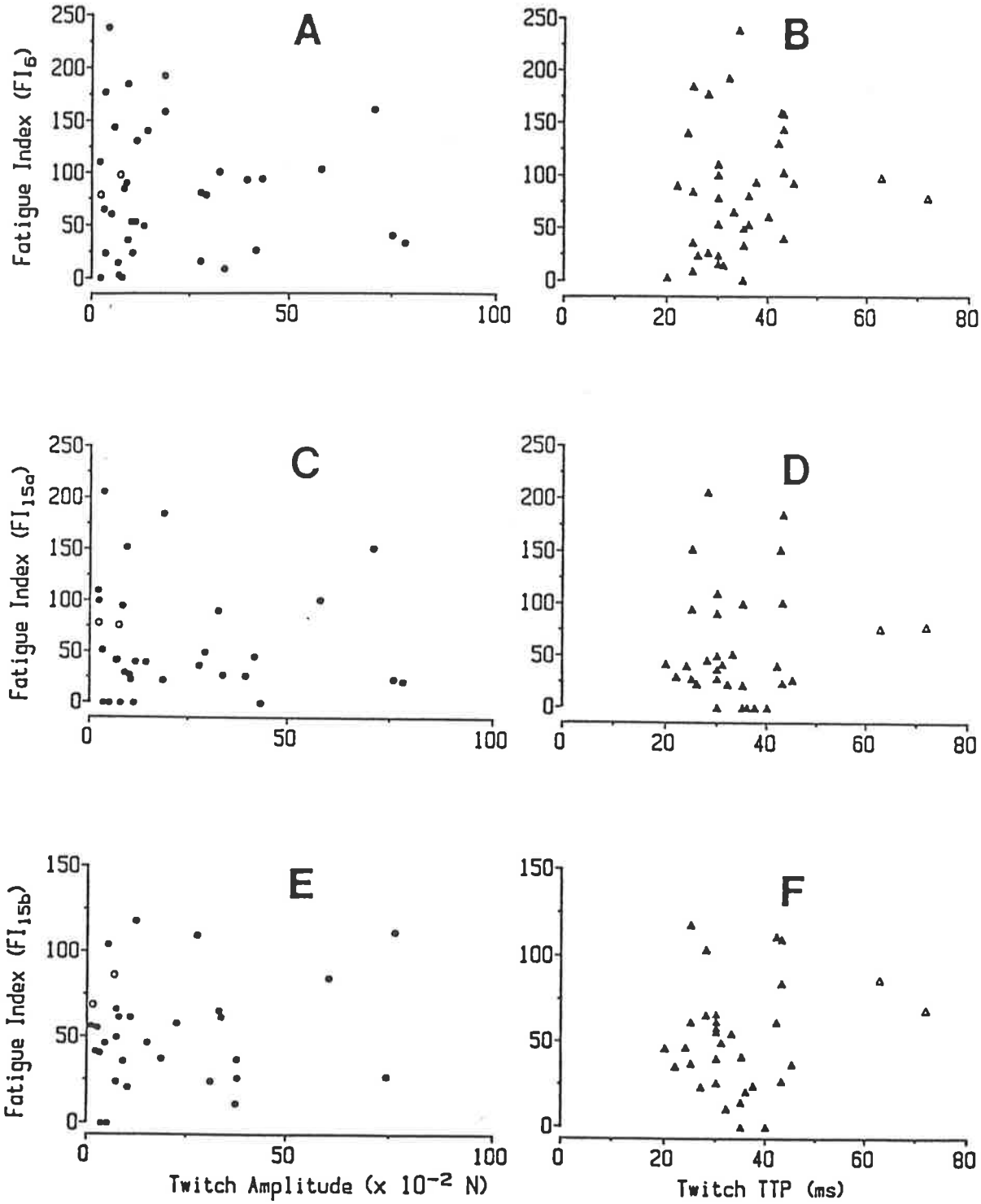

A
B
C
20
10
0
15
10
5
0
15
10
5
0
0
5
10
15
Twitch Amplitude (x 10⁻² N)
Time (min)

FIGURE 5.5

Pooled data showing the relationships between fatiguability and motor unit twitch amplitude and TTP. The relationship between initial twitch amplitude (calculated in the 1st minute) and FI_6 for 37 masseter units is shown in *A*, with the relationship between TTP and FI_6 for the same units shown in *B*. The relationship between initial twitch amplitude and FI_{15a} is shown in *C*, with TTP vs FI_{15a} in *D*. The twitch amplitude in the 1st 3 minutes of the contraction is plotted against FI_{15b} in *E*, with TTP vs FI_{15b} in *F*. The vertical scale in *E* & *F* is different from that in *A* – *D*. The values for two units with contraction times much slower than the main group are shown as open symbols in *A* – *F*.


A
B
C
D
E
F
Fatigue Index (FI_6)
Fatigue Index (FI_{15a})
Fatigue Index (FI_{15b})
Twitch Amplitude (x 10^{-2} N)
Twitch TTP (ms)
0
50
100
150
200
250
20
40
60
80

correlation coefficients for the relationships were −0.04 and 0.09, respectively. In general, the twitches showed more fatigue after 15 minutes than after 6 minutes. That is, the FI values are generally lower in Fig 5.5*C* than Fig 5.5*A*.

Fig 5.5*E* & *F* show the relationship between twitch amplitude and TTP with fatigue when 3 minute averaging epochs were used for the twitches and fatigue index (FI_{15b}). Similarly, there was no significant correlation between fatiguability and initial twitch amplitude (calculated over the first 3 minutes) or TTP. The use of 3 minute epochs for the twitch values allowed the frequently−observed potentiation of the twitch in the first few minutes of activity to be incorporated in the calculation of the fatigue index, and also reduced the scatter of the FI values.

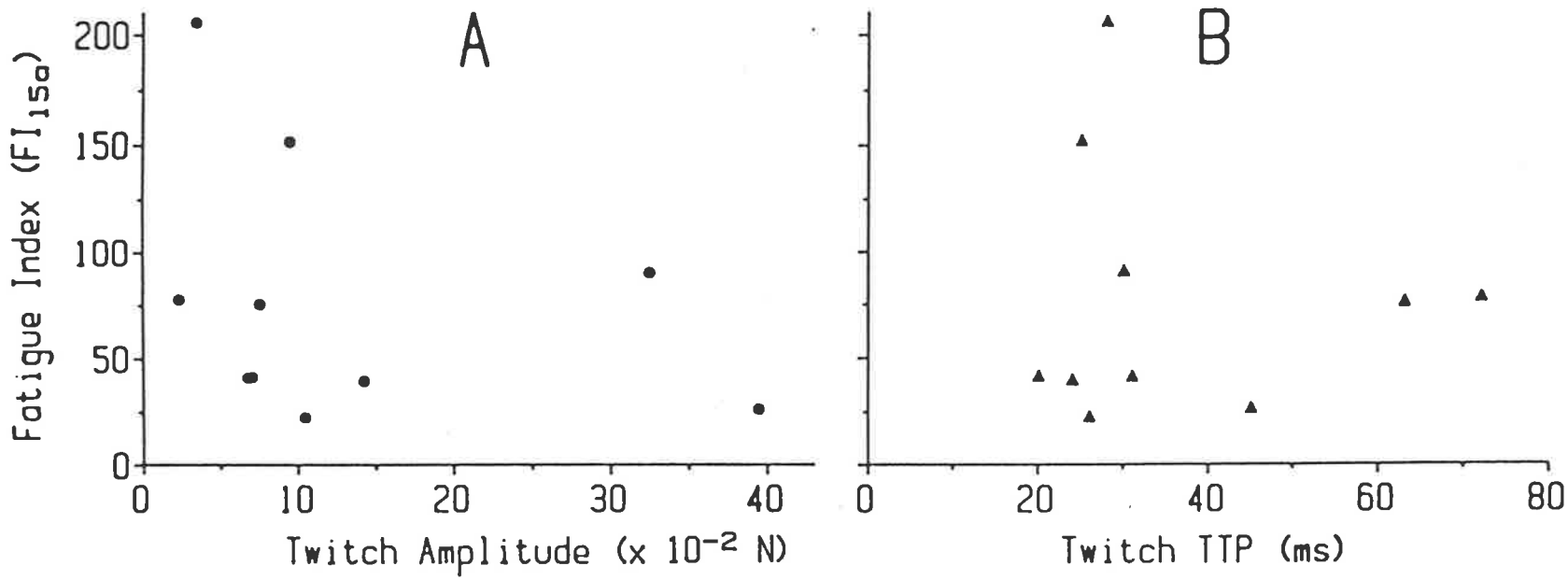
Regardless of the FI used, there was no significant correlation between fatiguability and motor unit twitch amplitude or TTP. Also, the distribution of FI for the units tested did not show obvious discontinuities which might have suggested a separation of the units into different physiological classifications. The values for two units with twitch TTP slower than 60 ms are represented by open symbols in Fig 5.5*A*−*F*. These units were both found in the same subject. Their twitch amplitudes were relatively small, and their various fatigue indices were all greater than 70, indicating that they were relatively fatigue resistant. These 2 units were the only units tested that might be classified as belonging to a separate group of small, slow, fatigue resistant units (Type S). The remaining units were fast−twitch, with a relatively continuous spectrum of fatiguability.

It might be argued that a relationship between motor unit size and fatiguability would be weakened by the pooling of data from a number of subjects, since individual variations in motor unit size and muscle strength and training undoubtedly introduced some scatter in the data points. This possibility was investigated in 5 subjects, in each of whom more than 4 units were fatigue tested for 15 min. In none of these subjects was there a significant correlation ($P < 0.05$) between either motor unit twitch amplitude, or TTP and the fatigue indices. The data for 10 units from one subject are presented in Fig 5.6.

During a voluntary contraction it is not possible to test all units under identical conditions, as in animal motor unit fatigue testing. For example, the total biting force at which each unit was tested was not constant for all units, and frequently changed during the course of the experiment. This could conceivably mean that units were tested under different local blood flow conditions. In the present experiments, the total biting force usually stayed relatively constant or fell during the period in which the motor unit was controlled at 10 Hz. In order to

FIGURE 5.6

The relationship between FI_{15a} and twitch tension and TTP for 10 units from one subject. The relationship between twitch amplitude and FI_{15a} (*A*) had a correlation coefficient $r = -0.31$, which was not significant ($P > 0.05$). The correlation coefficient (r) for the relationship between twitch TTP and FI_{15a} (*B*) was -0.07, which was also not significant ($P > 0.05$).


A
B
Fatigue Index (FI_{15a})
200
150
100
50
0
0
10
20
30
40
Twitch Amplitude (x 10^{-2} N)
0
20
40
60
80
Twitch TTP (ms)

investigate the effect of the total biting force level on motor unit fatigue, the activation threshold for each unit was expressed as a percentage of the MBF for the subject, and plotted against the unit's fatigue index. There was no significant correlation between this value and any of the fatigue indices used in this study. The relationship between the units' relative activation threshold and $FI15_a$ is shown in Fig. 5.7*A*. In *A*, there is a suggestion that the units recruited above 10% MBF were generally fatiguable (7 of 8 had an FI < 50), but units recruited at lower relative forces displayed a wide range of fatiguability, with the result that there was no significant correlation between activation threshold and fatiguability ($r = -0.05$; $P > 0.05$). A difference in fatiguability between the units recruited above and below 10% MBF was less evident when 3-minute epochs were used for the fatigue indices (Fig. 5.7*B*, $r = -0.01$; $P > 0.05$). It is concluded that masseter motor unit fatiguability was not strongly influenced by the relative strength of the contraction over the range 0–20% MBF. Over a similar range it would also seem that masseter motor units are not recruited in order of increasing fatiguability.

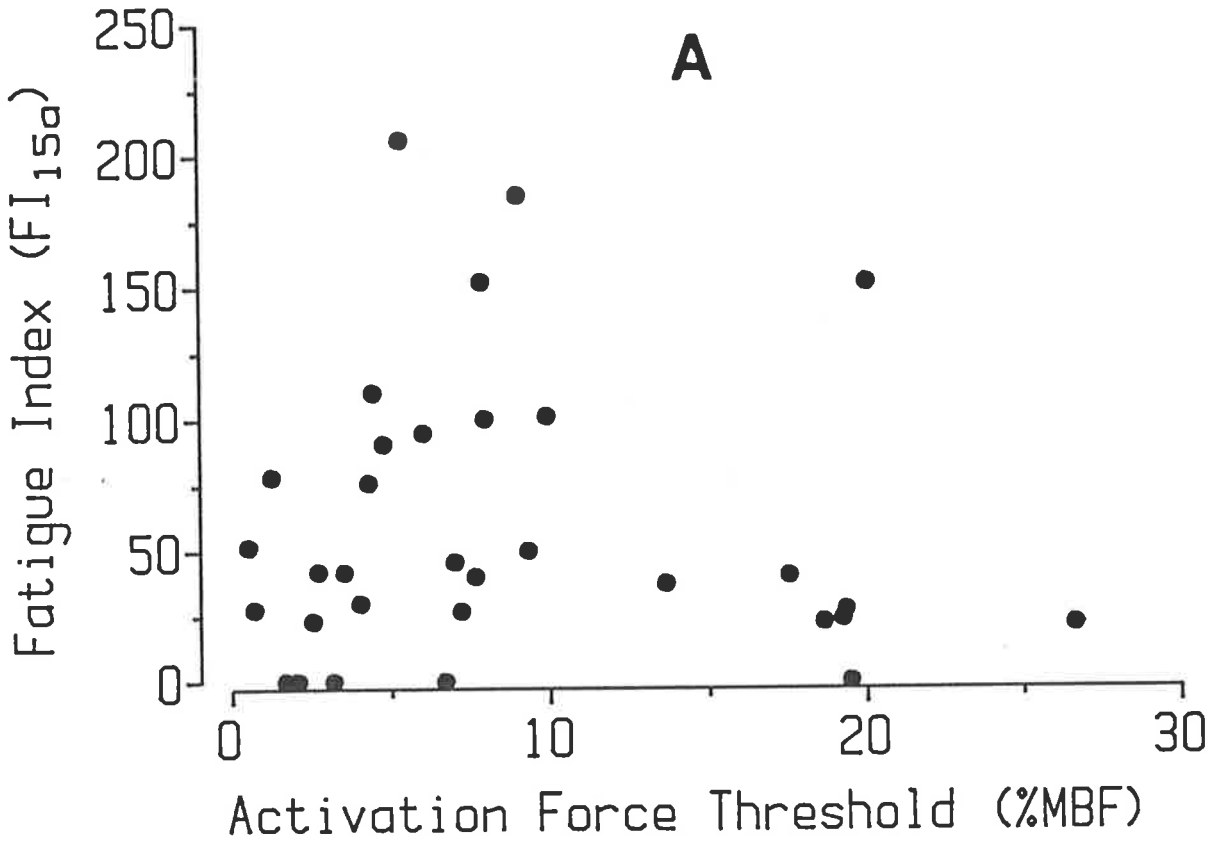
5.4 Discussion.

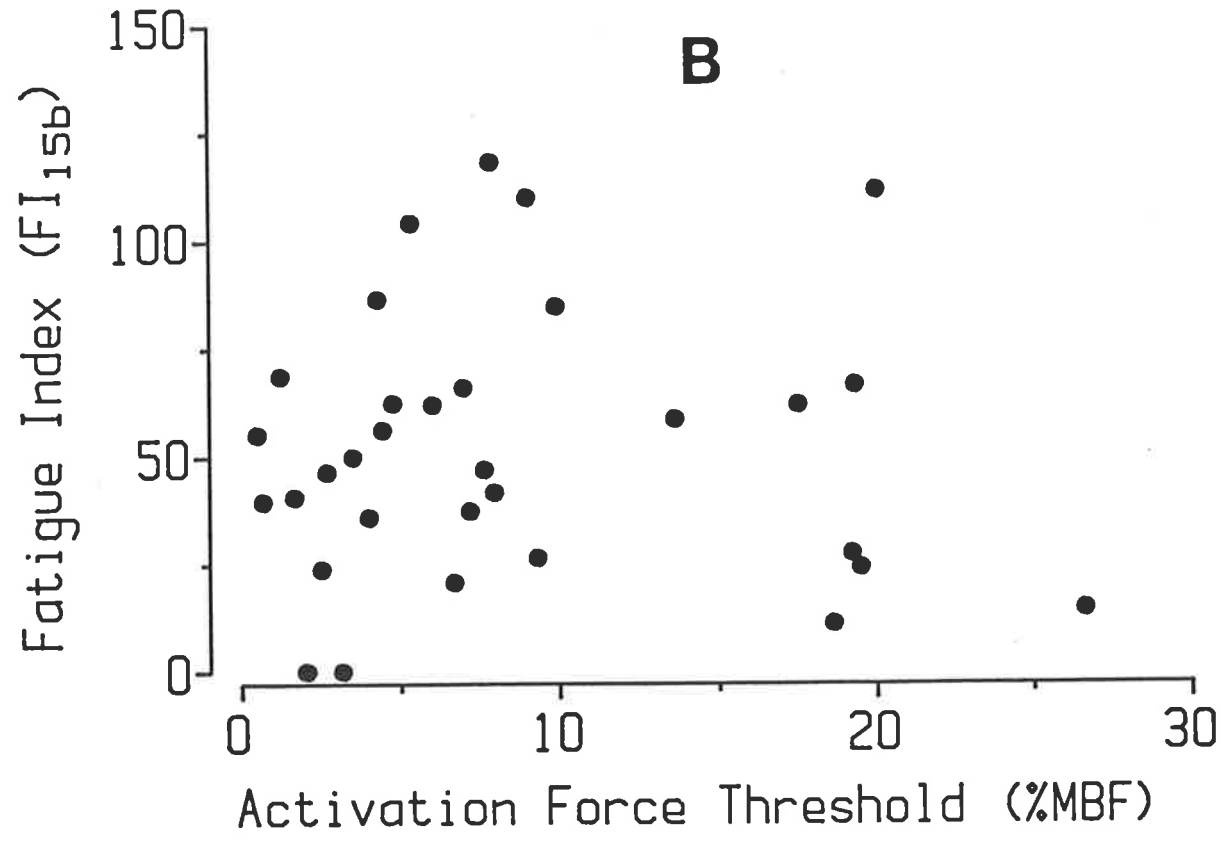
Previous studies in the masseter have reported a similar range of twitch amplitudes as those found in the present study, but a slower mean TTP. Yemm (1977*b*) reported a TTP range of 25–90 ms, with a mean around 56 ms and twitch amplitudes of 1–336 mN. Goldberg & Derfler (1977) found a TTP range of 38–69 ms, mean 49 $\pm$ 7.5 ms and twitch amplitudes of 110–2050 mN. The faster mean twitch TTP (34.8 $\pm$ 10.1 ms) in the present study may be due to differences in apparatus used, or perhaps due to the more stringent firing pattern parameters imposed.

There was a positive correlation between the unit's twitch amplitude and its voluntary activation threshold (Fig 5.1*A*; Fig. 5.3*A*). This relationship has been found in a number of muscles in humans, including the masseter (Yemm, 1977*b*; Goldberg & Derfler, 1977), and is in accord with the size principle of recruitment of motor units (Henneman & Mendell, 1981). The poor correlation between twitch tension and TTP of masseter motor units in the present study (Fig 5.1*B*; Fig. 5.3*B*) was also reported in both previous studies of human masseter motor units using STA. Although not a unique finding in human muscles (Sica & McComas, 1973; Young & Mayer, 1981), it is in contrast to the situation in most

FIGURE 5.7

The relationship between motor unit recruitment threshold and fatigue. The motor unit recruitment threshold (expressed as a percentage of the subject's MBF) is plotted against the unit's fatigue index after 15 minutes of activity. In *A*, the fatigue index was FI_{15a} (1 – minute epochs), and in *B* it was FI_{15b} (3 – minute epochs).


A
250
200
150
100
50
0
Fatigue Index (FI_{15a})
0
10
20
30
Activation Force Threshold (%MBF)


B
150
100
50
0
Fatigue Index (FI_{15b})
0
10
20
30
Activation Force Threshold (%MBF)

animal muscles studied, in which a negative correlation is found between motor unit twitch tension and TTP (Burke, 1981*a*).

Motor unit physiological and histochemical correlation.

The poor correlation of fatiguability with both motor unit twitch amplitude and contractile speed observed in the present study (Fig 5.5), together with the poor correlation between motor unit size and contractile speed, and the essentially unimodal distribution of relatively fast – twitch contraction times in human masseter observed in the present and previous studies (Yemm, 1977*b*; Goldberg & Derfler, 1977), suggest that the human masseter has very few small, slow, fatigue – resistant units, i.e., type S units. In the present study, only 2 units had contraction times that were substantially slower than the main group (Fig 5.5, open symbols). These units also had relatively small twitch amplitudes and were fatigue – resistant (FI's were all greater than 70). These were the only masseter units of those studied which might be classified as type S. In contrast, type S units form a substantial proportion of the unit population in many other muscles in which a physiological classification of the motor unit population has been performed (reviewed in Burke, 1981*a*).

In animals, the motor units classified physiologically as type S are the units whose muscle fibres also stain as type I with myosin ATPase (MATPase) (reviewed by Burke, 1981*a*). This has also been reported for human medial gastrocnemius (MG) (Garnett *et al.*, 1978). In the human masseter both type I and type II fibres are found in varying proportions, but with a predominance of type I fibres in most sites (Eriksson & Thornell, 1983). Despite the histochemical evidence that a substantial proportion of the masseter is comprised of type I fibres, the present findings reveal very few physiological type S units in this muscle. This is not unprecedented. A physiological classification of the motor unit populations of cat tibialis anterior (TA) and extensor digitorum longus (EDL) found them to be comprised, like the masseter in the present study, of mainly fast – twitch motor units with a wide range of fatiguability, despite histochemical evidence for a substantial proportion of type I fibres in both muscles (Goslow *et al.*, 1977). The human masseter generally lacks the type IIA fibres which are believed to correspond to the physiological type FR motor units (Eriksson & Thornell, 1983). However, a substantial population of fast – twitch, fatigue – resistant units (type FR) were found in the present study. The most likely explanation for the conflicting physiological and histological evidence is that at least some type FR units in human masseter have muscle fibres which stain histochemically as type I, which

suggests that the correlation between the physiological properties of motor units and the staining of their fibres for MATPase is not as strong as is presently believed.

There are no compelling reasons why the histochemical (MATPase) appearance of the muscle fibres, and the physiological properties of the motor units should be rigidly linked. It has been pointed out that the intensity of staining for MATPase is not an absolute indicator of contractile speed (Burke, 1981*a*). Perhaps factors other than the MATPase activity are important determinants of twitch speed in the masseter. There is also no direct relationship between fatigue resistance and the fibre type as determined by staining for MATPase. Although a relationship between histochemical fibre type and unit fatigue resistance has been demonstrated in the cat (Burke *et al.*, 1973), there is evidence that fatigue resistance correlates with the activity of oxidative enzymes, not MATPase activity *per se* (Nemeth *et al.*, 1981; Edström & Kugelberg, 1968; Kugelberg & Lindegren, 1979; Hamm *et al.* 1988). There can be a wide variation in oxidative enzyme activity among fibres of the same histochemical type (I or II) within human muscle (Lowry *et al.*, 1978), which makes it possible that the close correlation between MATPase staining and physiological motor unit type found in the cat may not apply in human muscles. The only study in humans where a direct comparison of motor unit physiological and histochemical properties has been performed is that of Garnett *et al.* (1978) in medial gastrocnemius. It is interesting to note that in that study, 1 of the 3 fast – twitch units whose fibre composition was determined was found to be associated with type I fibres.

It might be argued that only a small proportion of the masseter unit population has been activated in the present study, and that testing over the full range of activation thresholds may reveal a separate population of faster – contracting units. Even if this were the case, the population of units studied does not conform to the physiological type S classification, because of the wide range and extent of fatiguability found in the units. In rat soleus, type S units may respond to activation at 10 Hz for several hours with little loss of tension (Kugelberg & Edström, 1968). In human MG, all type S units lost less than 25% of contractile force after 3000 stimuli (Garnett *et al.*, 1978); this is quite different from the wide range of FI's after approximately 3000 activations in the present study (FI_6 in Fig 5.5).

The relationship between motor unit size, speed and fatiguability.

The relationship between motor unit size (i.e. peak tetanic tension or twitch tension), speed and fatiguability has been assessed in a number of studies in various muscles and species, including man (reviewed in Burke, 1981*a*). In general, there is a tendency for larger and faster – contracting motor units to be more fatiguable. The motor units in the present study do not conform to this pattern. There was no correlation between either motor unit twitch tension or TTP and the fatiguability of the unit (Fig 5.5). This unexpected result can be explained by consideration of the unique properties of the masseter motor unit population.

The first unusual feature of the masseter is the previously – discussed absence of a substantial population of physiological type S units. In heterogeneous muscles containing a mixture of type S, FF and FR units, the relationship between motor unit size, speed and fatiguability is due in large part to the pooling of data from the different motor unit types. Analysis within groups of units of the same physiological type weakens or eliminates these correlations. This was shown by Goslow *et al.* (1977) using data from cat MG, EDL and TA. In these cat muscles, when the type S population were excluded, a weakened but still significant correlation between motor unit size (peak tetanic tension) and fatiguability was found within the fast – twitch population. However, there was no significant correlation between twitch TTP and fatiguability in the fast – twitch units of TA and EDL, two muscles that had very few type S units. The motor unit profile of the human masseter is similar to these cat muscles, and the poor correlation between twitch TTP and fatiguability found in the present study supports the earlier suggestion that the association between contractile speed and fatiguability is not strong in muscles with predominantly fast – twitch motor units.

The poor correlation between motor unit twitch amplitude and fatiguability is also related to the poor correlation between twitch amplitude and TTP in the masseter units. Resistance to fatigue under aerobic conditions is correlated with the activity of mitochondrial enzymes in the muscle fibres; i.e. the ability to utilise oxidative means of ATP production (Kugelberg & Lindegren, 1979; Nemeth *et al.*, 1981; Hamm *et al.*, 1988). Logically, fatigue resistance is also related to the rate at which ATP is consumed. If the metabolic needs exceed the rate of supply, force falls. The rate of ATP consumption is believed to be directly proportional to the force produced (Dawson *et al.*, 1978) and to speed of contraction (due to more cross – bridges cycling per unit time). Therefore low – force, slow units have

a double advantage in terms of fuel economy. In most muscles, the low – force units are also usually slow (i.e. type S), and together with their high oxidative capacity are thus highly resistant to fatigue. In the masseter, there is no correlation between unit twitch size and speed; Yemm (1977*b*) even found a tendency in some individuals for larger units to be slower. This peculiar combination of motor unit size and speed will tend to reduce the difference in the rate of ATP consumption between large and small units in the masseter, compared with other muscles. It follows from this that the relationship between twitch force and fatiguability will be less clear in the masseter than in muscles with a negative correlation between motor unit twitch size and TTP.

The muscle fibre architecture in the human masseter also has several unique features which further complicate the relationship between motor unit size and fatiguability. The type I fibres in the masseter have a larger fibre diameter than that of the type II fibres in nearly all sites in the muscle (Ringquist, 1973*b*; Serratrice *et al.*, 1976; Vignon *et al.*, 1980; Eriksson & Thornell, 1983). This is in contrast to the limb muscles where the type I and II fibres are either of similar size, or the type II fibres have a larger diameter (Dubowitz & Brooke, 1973). Furthermore, the diameter of both type I & II fibres in the masseter (and other masticatory muscles) is generally smaller than the corresponding type in other skeletal muscles (Vignon *et al.*, 1980; Ringquist, 1971; Polgar *et al.*, 1973; Eriksson & Thornell, 1983). Small fibre diameter is believed to be associated with a reliance on aerobic metabolism, and the need to minimise diffusion distance of the blood supply. If this is correct, type II fibres in the human masseter would have an anatomical advantage over type I fibres in utilisation of blood – borne energy sources, and both types would be anatomically better suited to this purpose than the corresponding fibres in other muscles. In the muscles in which a direct comparison has been made, Type II fibres are associated with larger – twitch motor units (Burke *et al.*, 1973). If this relationship also applies in the masseter, the relatively smaller diameter of type II fibres in the masseter would optimise the ability of these fibres to utilise aerobic sources of energy, and may help to explain the poor correlation between motor unit twitch size and fatiguability found in the present study. If, however, the type II fibres in human masseter belong to faster, but not necessarily larger – twitch motor units (a notion supported by the poor correlation between motor unit twitch amplitude and TTP), then the relation between unit size and fatiguability would be expected to be even less clear.

Finally, there is a wide spectrum of activity of oxidative enzymes among the different fibre types in human masseter. Mitochondrial oxidative enzyme activity

(assessed by the staining for NADH – tetrazolium reductase) ranges from weak to very strong in type I fibres; from weak to strong in type IM, type IIC, and type IIA fibres; and from very weak to strong in type IIB fibres (Eriksson & Thornell, 1983). That is, some type I fibres stain weakly for oxidative metabolism, while some type II fibres may stain strongly. Even if type I fibres in the human masseter belong to units with smaller twitch tensions than those associated with type II fibres (this remains uncertain), the wide and overlapping range of oxidative enzyme activities in the different fibre types would lead to further variation in the relationship between twitch tension and fatiguability. The expected relationship between unit size and fatiguability would be further clouded if the type I fibres in the human masseter belong to slower, but not necessarily smaller – twitch motor units (which is one interpretation of the physiological data).

Comparison with other human motor unit fatigue data.

A comparison of the present data with the three other human motor unit fatigue studies is presented in Table 5.1. In each case, the unit populations were grouped on the basis of their fatigue indices. The three categories were FI > 75 (fatigue – resistant); FI between 25 and 75 (intermediate fatigue); and FI < 25 (highly fatigued). The values in each column represent the percentage of units falling within each category with a particular fatigue test. The first 3 columns show the data for the 3 fatigue indices used for the masseter units in the present study.

TABLE 5.1

DISTRIBUTION OF FATIGUE AMONG MOTOR UNITS IN SEVERAL HUMAN MUSCLES

	FI_6	FI_{15a}	FI_{15b}	@	#	$
FI > 75	56	34	19	62	90	55
FI 25 – 75	28	38	59	24	7	39
FI < 25	16	28	22	14	3	6

FI_6, FI_{15a}, FI_{15b} are the fatigue indices used in the present study.
@ = Human first dorsal interosseous (FDI); Stephens & Usherwood, 1977.
# = Human FDI; Young & Mayer, 1981.
$ = Human medial gastrocnemius (MG); Garnett *et al.*, 1978.

In the study by Stephens & Usherwood (1977), STA was used to assess twitch fatigue of 22 motor units in human first dorsal interosseous (FDI) after 5 minutes

of continuous activity at 10 Hz (approximately 3000 firings). This is best compared with FI_6 in the present study. It is also worth noting that both studies tested units at comparable initial total force levels, expressed as a percentage of maximal. In the present study 75% of tested units had an activation threshold below 10% of maximal force, while in the FDI approximately 63% of the tested units had an activation threshold below 10% of maximal. The distribution of FI's after 5 minutes of activity was similar in the FDI and masseter units.

In a study of human FDI motor units by Young & Mayer (1981), intramuscular microstimulation was used to activate single units, and a FI was found for 41 motor units. The fatiguing paradigm was 30 Hz pulse trains for 330 ms, repeated once per second for 2 min. This resulted in about 1200 stimuli being delivered. This test paradigm was chosen to be similar to the standard fatigue test which has been widely used to categorise the motor unit populations of a number of animal muscles (for review see Burke, 1981*a*). However, it would seem that 2 minutes of stimulation is insufficient to induce contractile fatigue in this muscle, as most units were not fatigued by the test regimen. In fact, 83% of the units were potentiated by the test (FI > 100). Two minutes is also insufficient for the masseter, as many units in the present study were potentiated by continuous activity at 10 Hz during the first 2 – 4 minutes.

The only other fatigue data for human motor units is in the MG muscle (Garnett *et al.*, 1978). Intramuscular microstimulation was used to fatigue 18 units by activation at 10 – 20 Hz for 0.5s, repeated every 1 or 2s. The FI was taken as the ratio of the initial twitch or tetanic tension to that after 3000 stimuli, which is comparable to FI_6 in the present study. Once again, the distribution of motor unit fatiguability was similar to that seen in the masseter.

This comparison suggests that pattern of contractile fatigue in masseter motor units is similar to that seen in FDI and MG after about 3000 activations. It has been suggested that the human masseter is more resistant to fatigue than the limb muscles during repeated maximal efforts (van Steenberghe *et al.*, 1978; Clark *et al.*, 1984; Clark & Carter, 1985). Although different factors undoubtedly influence fatiguability in maximal and low – force isometric contractions, the present findings suggest that superiority of the masseter in repeated high – force contractions is not due to an inherent advantage in aerobic fatigue resistance of its low – and moderate – threshold motor units. Therefore, other explanations, such as superior oxygenation (van Steenberghe *et al.*, 1978), become more likely. Evidence supporting this view include the high capillary density (Taylor *et al.*, 1973) and

small fibre diameter of the masticatory muscles (Eriksson & Thornell, 1983; Polgar *et al.*, 1973; Ringquist, 1971; Vignon *et al.*, 1980), and the observation that blood flow in human masseter is not significantly reduced during isometric clenching at levels of up to 50% MBF, compared with pre – contraction resting levels (Rasmussen *et al.*, 1977).

In the only other human motor unit fatigue study using voluntary activation there was a tendency for FDI units recruited at higher total force levels to fatigue more than units for which the total force level was low during the test (Stephens & Usherwood, 1977). Although the range of relative forces over which units were tested was greater in the FDI study (0 – 58% MVC), the majority of units tested in the FDI were recruited at comparable levels to those found in the present study (compare Fig. 5.7 with Fig. 4*B* of Stephens & Usherwood, 1977). Even at low forces in the FDI, there was a clear relationship between motor unit recruitment threshold and fatiguability (Fig. 4*A* of Stephens & Usherwood, 1977). Over a similar range of relative forces in the present study (0 – 20% MBF), no correlation was found between recruitment threshold and fatiguability for the masseter units (Fig. 5.7).

The interpretation of these differences is complicated by the problem that in voluntary contractions the units are not tested under identical conditions in terms of total force. The greater fatiguability of the high – threshold units in FDI may reflect differences in the inherent properties of the motor units recruited over this range of forces in the FDI, but the same result could be found if the higher – threshold units in FDI had a similar oxidative capacity to the low – threshold units, but at higher forces the blood supply became progressively more occluded. The poor correlation between recruitment threshold and fatiguability found in the masseter units tested over a similar range of forces may simply reflect a better preservation of blood flow in the masseter at higher forces, as suggested by the xenon clearance studies of Rasmussen *et al.* (1977). Alternatively, it may reflect differences in the pattern of change in the total force in the two muscles during the task of controlling the firing rate of one motor unit at 10 Hz. A more rapid or pronounced fall in total force in the masseter experiments might help to preserve blood flow and minimise fatigue. The total force usually fell during the masseter experiments, but this was not a universal finding (see Chapter 9). The importance of the force level at which the unit is tested (as distinct from the recruitment force) was alluded to by Stephens & Usherwood (1977), but they did not comment on the stability of the total force with time in their FDI experiments.

Regardless of the explanation for the poor correlation between biting force and motor unit fatiguability, the evidence suggests that under conditions of voluntary isometric incisor biting in the range 0 – 20% MBF, masseter motor units are not recruited in order of decreasing fatigue resistance.

Why should there be a poor correlation between motor unit recruitment threshold and fatiguability in the human masseter? From a functional viewpoint, the human masseter is quite different to the limb muscles, both animal and human, in which virtually the entire body of motor unit fatigue data has been collected to date. The masseter has minimal tonic postural activity. Support of the jaw against gravity is achieved by passive tension, with perhaps some activity in temporalis motor units (Yemm, 1976). At rest, no unit activity can be detected in the masseter. The paucity of type S units in the masseter is therefore not surprising. The correlation between small size of the motor unit and resistance to fatigue is usually explained as being related to the fact that smaller units are active for longer periods during muscle contraction (a consequence of the size principle of recruitment) and therefore this higher level of activity trains the oxidative capacity of the muscle fibres to a greater extent than the less frequently – active high – force units. In limb muscles, the motor unit activity patterns during normal usage would tend to support this concept (Hennig & Lømo, 1984). However, as previously mentioned, the human masseter does not have a tonic postural role, but is involved in a wide variety of activities which include mastication, speech, swallowing, and facial expression. These activities generally require rapid, intermittent activation of the muscle. Under these conditions the recruitment of motor units becomes compressed (Büdingen & Freund, 1976), and it is possible that masseter units with differences in recruitment thresholds during slow force ramps comparable to the range found in the present study may have similar activity patterns (and hence "training") during normal usage of this muscle.

In summary, it is apparent that motor units in the human masseter possess combinations of motor unit physiological, histochemical, and anatomical properties which differ from the relationships widely accepted for animal limb muscles. One can only speculate that the unique features of the human masseter reflect its complex functional requirements.

CHAPTER 6.

CHANGES IN THE CONTRIBUTION OF SINGLE MOTOR UNITS TO THE SURFACE ELECTROMYOGRAM DURING A FATIGUING VOLUNTARY CONTRACTION.

6.1 Introduction.

Under experimental conditions, fatigue can perhaps be defined most clearly as the loss of force which occurs while a muscle is receiving a constant level of excitation. In animal experiments it is a relatively simple matter to measure fatigue under conditions of constant excitation by electrically stimulating either the whole muscle or single motor units and recording the force changes. In voluntary contractions of whole muscles in humans, a constant excitation approach requires that the same units are active throughout the contraction, and that their firing pattern does not change in a manner that affects the force output. In whole muscles, these criteria are only satisfied during a maximal voluntary contraction (MVC), where maintenance of constant (maximal) excitation is verified by intermittent supra – maximal nerve shocks (e.g., Bigland – Ritchie, 1981*a*).

The difficulty in verifying a constant level of excitation of muscles in voluntary submaximal contractions in humans has resulted in a more indirect approach to fatigue testing in this situation. The usual procedure is for the subject to maintain the force at a constant submaximal level, and changes in the surface electromyogram (EMG) are monitored (e.g., Edwards & Lippold 1956; Maton, 1981; Häkkinen & Komi, 1983; Lindström & Hellsing, 1983). The limitation of a constant – force paradigm is that fatigue cannot be objectively measured in terms of loss of force, but must be inferred from changes in the surface EMG signal. The relationship between force and the surface EMG is complex, and not completely understood, which raises the possibility that under some conditions fatigue (or lack of) may be misinterpreted from the surface EMG data.

Many of the problems inherent in whole – muscle, submaximal fatigue tests are avoided when fatigue is examined at the level of the motor units. In the preceding Chapter contractile fatigue was assessed in masseter single motor units using a constant – excitation paradigm (continuous activation at 10 Hz). In the

present Chapter, the relationship between motor unit contractile fatigue and changes in the unit's contribution to the surface EMG signal is examined, using the same paradigm. This approach has the advantage that both EMG and force parameters are measured under conditions of constant excitation, in which case fatigue can be directly measured in terms of force loss. This allows a direct comparison between changes in the EMG signal and contractile fatigue. These data are useful in identifying the likely causes of force loss in this situation, and in assessing how well changes in the EMG signal correlate with an objective measure of contractile fatigue. The significance of these findings at the motor unit level are discussed in terms of the use of whole muscle surface EMG in human submaximal fatigue studies.

6.2 Methods.

Apparatus and recording procedure.

These arrangements were identical to those described in detail in Section 5.2. The units included in the present study were a subgroup of those included in Chapter 5.

Protocol.

This was also identical to that used in Chapter 5. The subject controlled the mean firing rate of a selected masseter single unit at 10 Hz for 15 minutes during a continuous isometric contraction, with the aid of visual and audio feedback.

Analysis.

Single unit spike trains.

The intramuscular EMG records were analysed off-line to determine the firing pattern of the single unit(s) recorded during the contraction, and to generate trigger pulses for the averager. Single motor units were discriminated using either an amplitude discriminator, or the SPS 8701. The interspike intervals (ISI's) in the train of spikes from each unit were stored on disk.

Test for constant firing rate (excitation).

The required firing rate was 10 Hz (mean ISI of 100 ms). The mean ISI $\pm$ standard deviation of the discharge of the unit controlled by the subject was

calculated for each 30s epoch throughout the test by a special-purpose computer program from the intervals stored on disk. The values from each epoch were tested for a significant deviation from the required value of 100 ms with a t-test. As would be expected, since the subject was voluntarily controlling the rate, it was extremely rare for the mean ISI in any epoch to be significantly different from 100 ms ($P < 0.05$).

Spike-triggered Averaging.

Tests for independence of motor unit discharge.

The methods used to assess masseter motor unit synchronization and correlated activity have been described in full in Chapter 2. The Milner-Brown test was performed for all units during the initial and final 3-minute epoch. Units exhibiting evidence of synchronous or correlated activity using the criteria of Milner-Brown *et al.* (1975) were not included in the present study. No units were found which passed the Milner-Brown test for synchrony in the first epoch, but subsequently failed in the final epoch.

Single motor unit twitches.

The method of determining single motor unit twitches using STA was identical to that described in Chapter 5. The normal epoch duration in the present study was 1 minute. Spike parameters 300-100:100-300 were used as valid triggers for the averaged twitches. The stringent firing pattern constraints imposed resulted in less than 1 in 5 firings of the single unit being accepted as a valid trigger; about 100 triggers were used to produce the averaged twitch records in most 60s epochs.

The resulting single motor unit twitches (mean $\pm$ SEM) for each epoch were plotted. The peak twitch amplitude $\pm$ SEM, and the twitch time-to-peak (TTP) were measured from the plots. The change in the twitch amplitude between the first and 15th minute of the contraction was expressed as a twitch Fatigue Index (FI), which was calculated according to the following formula: (twitch amplitude from 15th minute / twitch amplitude from 1st minute) x 100.

The surface representation of the single motor unit potentials.

The contribution of each motor unit action potential to the surface EMG signal was determined using STA (Yemm, 1977*a*). The surface EMG record was filtered in the bandwidth 2-500 Hz before averaging by the computer (PDP 11/73, sampling rate 1000 Hz per channel). The surface EMG was averaged over the same epochs as the force records, although all of the unit's action potentials were

accepted as triggers (in contrast to the stringent constraints on triggers used for the twitch averaging procedure). The surface representations for each epoch were plotted in the same manner as the twitch records. The records usually contained a prominent positive and negative peak from which the peak – to – peak amplitude was measured (Fig. 6.1). With the usual number of around 600 triggers in the average the standard error of the peak – to peak amplitude of the surface representation was small. The peak – to – peak amplitude of the surface representation in the 15th minute was compared to that from the 1st minute and the result expressed as a percentage change. The duration of the unit's surface representation waveform was assessed by measuring the temporal relationship of prominent peaks in the waveform. In order to improve the resolution of the duration measurements for quantitative analysis, the surface EMG from each minute of the contraction was re – averaged (512 counts) using a "black box" averager (Neurolog NL 750) with an effective sampling rate of 5 kHz. The output of the averager was displayed on a storage oscilloscope and the change in duration measured. By this means changes in duration as small as 2% could be detected. Averaging with this procedure (sampling rate 5 kHz) revealed an identical pattern of change in the *amplitude* of the surface representation of the unit as did averaging the same signal with the PDP 11/73 (sampling rate 1 kHz), which confirmed that the lower (1 kHz) sampling rate was acceptable for measurement of the surface representation amplitude.

Action Potentials from the intramuscular records.

Because of the higher frequency content of the intramuscular EMG signal and the sampling rate limitations of the averaging computer these records were averaged off – line using the Neurolog NL 750 averager (effective sampling rate 25 kHz). The intramuscular EMG signal was filtered (50 – 2500 Hz) and passed through an analog delay device (NL 740) prior to averaging in order to see the entire waveform in the average. Average single unit intramuscular action potentials were made from the first and 15th minute of the contraction, using 64 triggers for each average. The change in peak – to – peak amplitude between the first and fifteenth minutes was expressed as a percentage. All units were scrutinised carefully during the experiment to ensure that any changes in action potential waveform were seen to occur slowly and progressively in order to be confident of the identity of the unit.

Total biting force.

The activation threshold force of the single motor units was determined off-line from the force ramps. The total biting force was plotted against time for the 15 minutes. The relative strength of the contraction was judged by comparison with each subject's maximal biting force (recorded on a previous occasion).

6.3 Results.

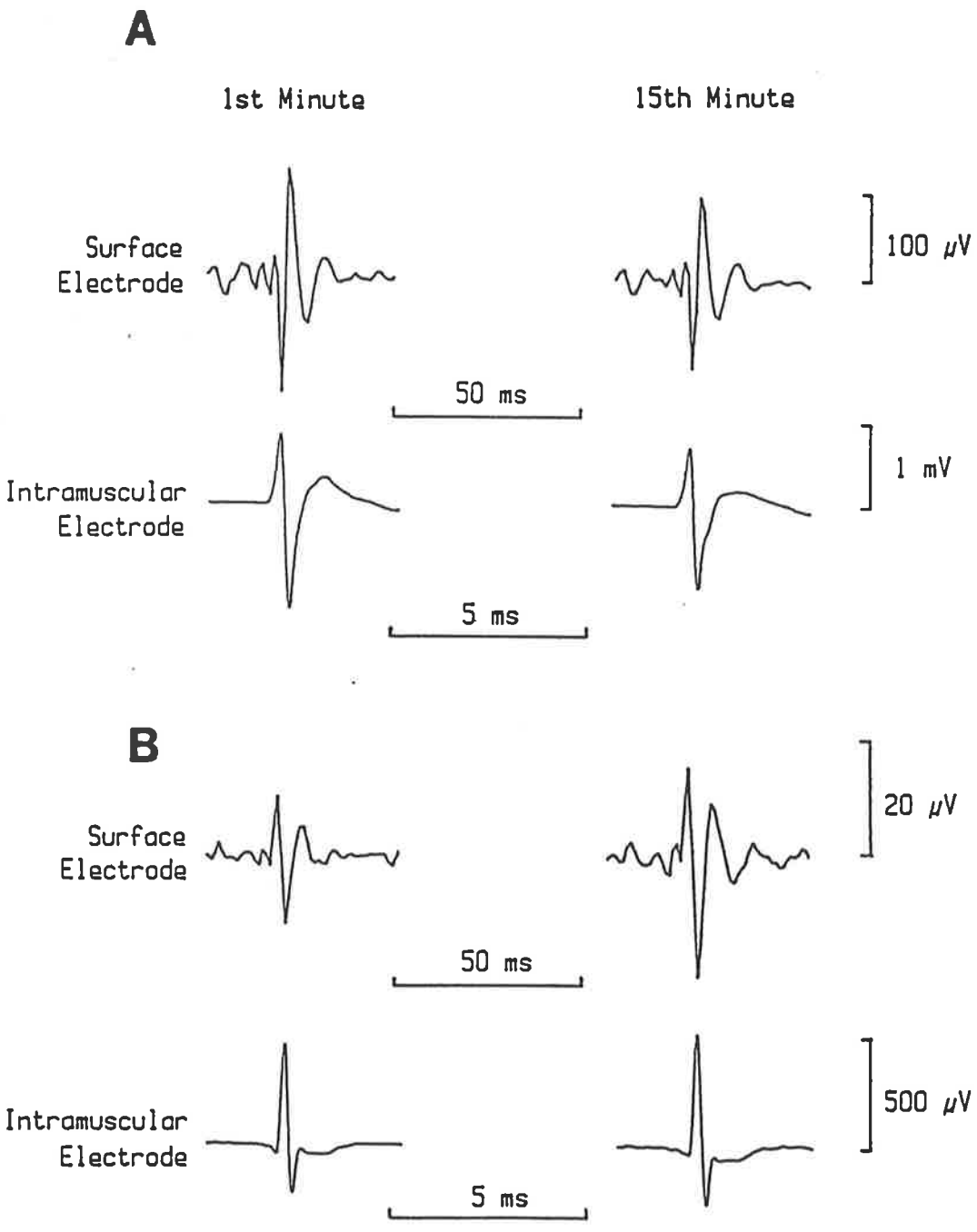
A total of 18 single motor units recorded in 12 separate experimental sessions from 8 individuals were included in the present study. The amplitude of the motor unit action potentials detected with the intramuscular fine wire electrodes ranged from 126 to 1600 μV (mean 605 μV, n=18). The amplitude of the surface representation of the single motor unit potentials ranged from 10 μV to 234 μV (mean 62 μV, n=18). On most occasions additional units could be discriminated as well as the unit controlled by the subject at 10 Hz. The surface representations were determined for these units wherever possible. Since they were not firing at a constant mean rate of 10 Hz the data from these units were not included in the main study, however information from these concurrently active units was useful in considering the possible causes of some of the observations.

The surface representation of the motor unit potential was not constant over the 15 minute contraction. The most striking change was in the amplitude of the surface representation. This ranged from an increase in amplitude in one unit of 100% to a decrease in amplitude in another of 53%. Most units showed some change in the amplitude of the surface representation with time. Two units representative of the range of amplitude changes are shown in Fig. 6.1. The Unit *A* in Fig. 6.1 is an example of a unit which showed a decrease in the amplitude of the surface representation with time, while Unit *B* is a unit whose surface representation increased in amplitude after 15 minutes. The average intramuscular potential of the unit in the corresponding epoch is shown immediately below each surface record.

The twitch tension of most units decreased over the 15 minutes. The averaged twitches of 3 units, recorded from different subjects, from the first and 15th minute of continuous activity are shown in Fig. 6.2 (left hand side). The units marked *A* and *B* are the same units whose EMG records are depicted in Fig. 6.1. On the right side of Fig. 6.2 the twitch tension (filled circles) for the

FIGURE 6.1

The change in the surface representation of two different single units during the 15 – minute contraction. The upper traces are the representation of the unit potential in the surface EMG signal, obtained by STA during the first and 15th minute of the contraction. The surface averages were typically obtained using about 600 triggers. The traces directly below show the action potential recorded with the intramuscular electrode during the corresponding epoch (average of 64 counts). In Unit *A*, the amplitude of the surface potential (upper traces) had decreased by the 15th minute, whereas in Unit *B* the amplitude of the surface representation had increased. Note that the duration of the surface potential waveforms was quite stable. Similar changes were seen in the intramuscular action potentials of these two units, but the intramuscular records were generally not reliable for quantitative analysis (e.g., Fig. 6.7).


A
1st Minute
15th Minute
Surface Electrode
100 μV
50 ms
Intramuscular Electrode
1 mV
5 ms
B
Surface Electrode
20 μV
50 ms
Intramuscular Electrode
500 μV
5 ms

corresponding unit is plotted at 1 minute intervals throughout the contraction. There was a tendency for a progressive reduction in twitch amplitude with time, and in each unit, the twitch had fatigued significantly by the 15th minute (paired $t-$test, $P<0.05$). Of the 18 units included in this study; in 12 the twitch had fatigued significantly after 15 minutes ($P<0.05$), in 5 units the twitch was not significantly different ($P>0.05$), and in 1 unit the twitch increased significantly ($P<0.05$).

Several points regarding the duration of the surface representation waveform are illustrated in Fig. 6.2. First, in all 3 units shown the duration of the surface representation (open squares) was very stable during the contraction. For the 18 units studied, the surface representations of 10 changed in duration by less than $\pm 5\%$ after 15 minutes. Only one had changed by more than 20% over this period. Second, the duration of the surface representation waveforms of the units in Fig. 6.2 remained relatively constant while the twitches fatigued significantly. The pooled data from all units showed that the twitch FI was independent of the change in duration of the surface potential of the unit after 15 minutes ($r=0.01$, $P>0.05$). There was also no significant correlation between the change in duration of the unit surface potential and its change in amplitude ($r=0.06$, $P>0.05$).

The pattern of changes in amplitude of the surface representation waveform with time shown in Fig. 6.2 was typical. The amplitude (filled triangles) was fairly stable from one epoch to the next, and in those units which displayed a large change in amplitude over the 15 minutes the trend was usually gradual and evident over a number of epochs (Fig. 6.2*A* & *B*; filled triangles). The changes in amplitude of the surface representation were not related in a consistent manner to the contractile state of the motor unit. In each unit shown in Fig. 6.2 the twitch had fatigued significantly by the 15th minute, yet in *A* the surface representation amplitude had decreased, in *B* the amplitude had increased and in *C* it was relatively unchanged. Indeed, in *B* after 10 minutes the twitch had fallen to zero, yet the amplitude of its surface representation in this period was virtually unchanged.

The relationship between the unit's twitch FI and its change in surface representation amplitude for the 18 units is shown in Fig. 6.3. There was no significant correlation between these two variables ($r=0.06$, $P>0.05$). The correlation coefficient for these data was influenced strongly by the two units whose surface representation amplitudes increased markedly with time. The data

FIGURE 6.2

Time – dependent changes in twitch tension and surface representation waveform for 3 masseter units. The twitch records of three units *A*, *B*, & *C* from the first and 15th minute of continuous activity are shown on the left. The arrows mark the time of occurrence of the trigger. Each average is the result of about 100 counts. The force calibration is 200 mN for *A* & *C*, and 40 mN for *B*. The horizontal calibration is 50 ms. Fatigue is evident in the twitches from each unit at 15 minutes. On the right side the twitch tension (filled circles), surface representation amplitude (filled triangles), and surface representation duration (open squares) for the corresponding unit is plotted for each 1 – minute epoch over the 15 minutes. Each point has been normalised as a percentage of the value in the 1st minute. Error bars on the twitch tension values at 1 and 15 minutes represent 1 standard error of the mean.

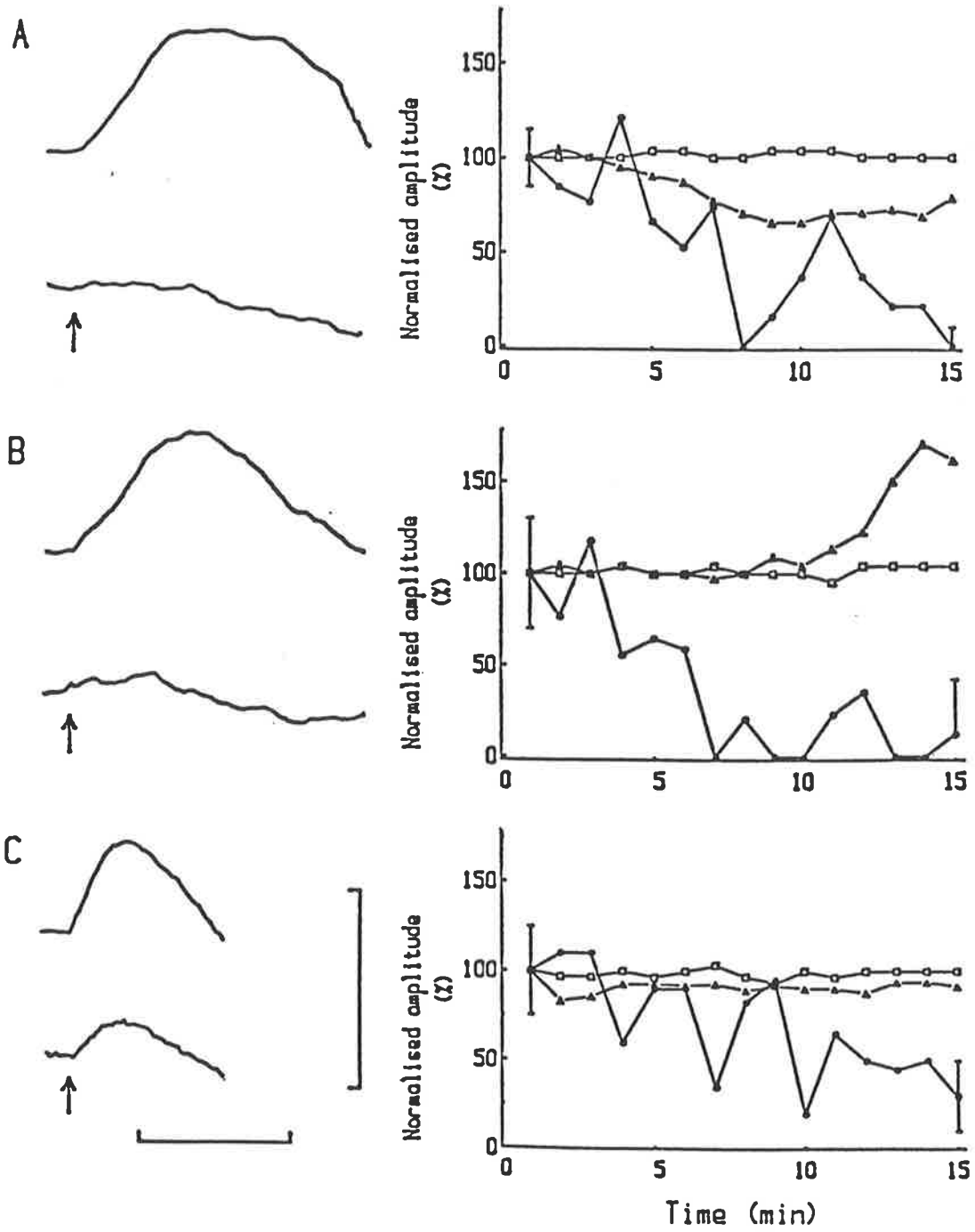
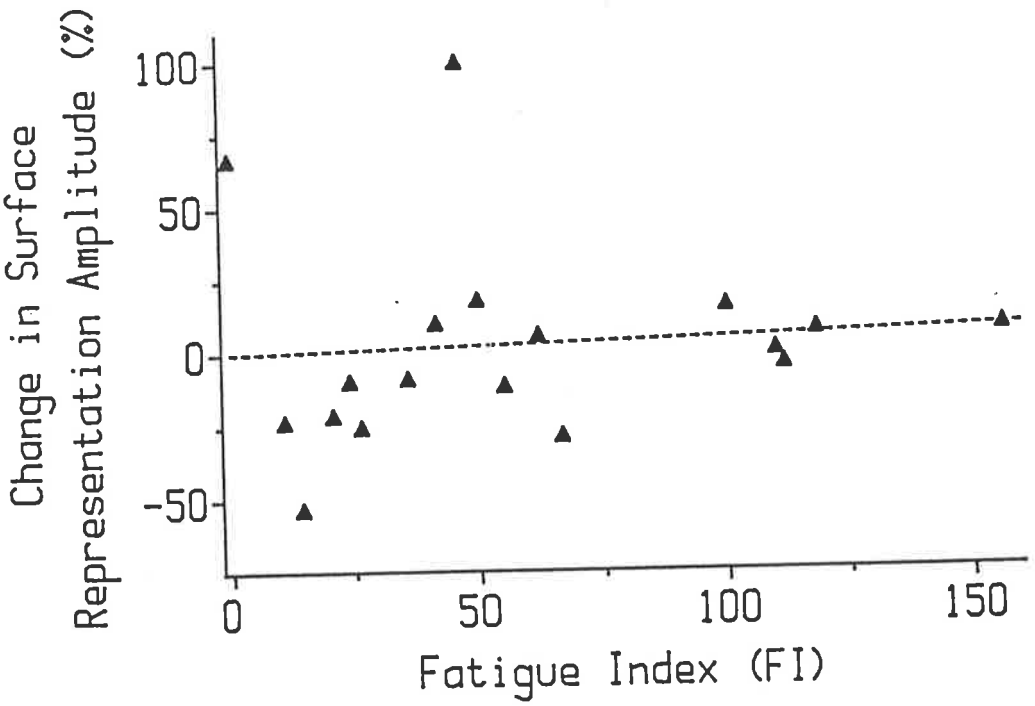

A
B
C
Normalised amplitude (%)
150
100
50
0
0
5
10
15
Time (min)

FIGURE 6.3

The relationship between the change in amplitude of the surface representation of the single unit and the twitch fatigue index. The dashed line is the linear regression line of best fit. The correlation co – efficient (r) was 0.06, which indicates that there was not a significant correlation between these two variables ($P > 0.05$).


Change in Surface
Representation Amplitude (%)
100
50
0
-50
0
50
100
150
Fatigue Index (FI)

from these two units were carefully re-checked for possible artifacts, such as false triggers from faulty action potential recognition, or synchronization-related errors, but none were found.

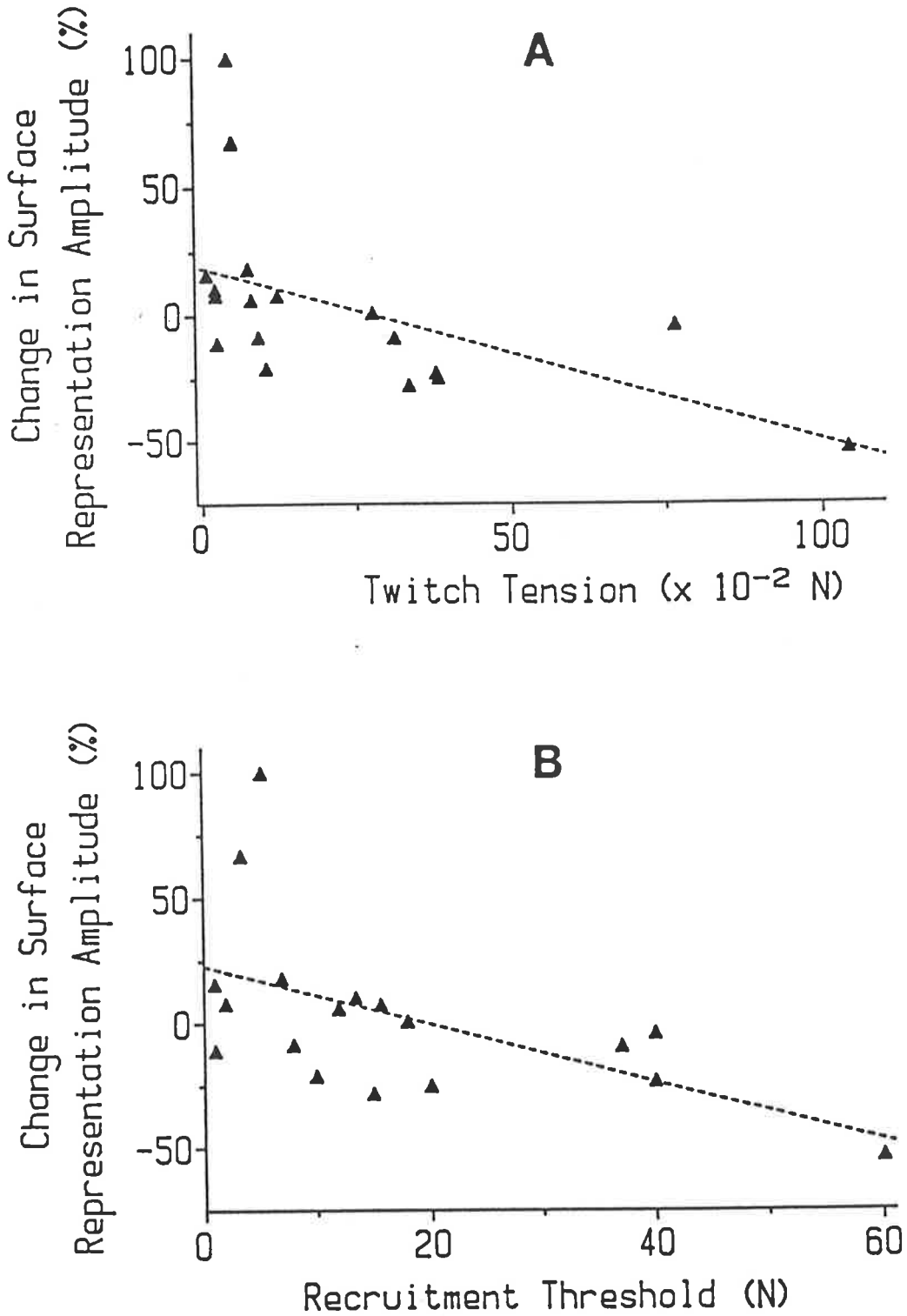
There was a relationship between the size of the motor unit and the change in amplitude of its surface representation with time. Fig. 6.4*A* is a scatter diagram of the initial twitch tension of the units against the change in amplitude of their normalised surface representations. There was a tendency for the amplitude of the surface representation to increase in the small units and to decrease in the larger units over the 15 minutes. The negative correlation between twitch tension and the change in the surface representation was significant ($r = -0.55$, $P < 0.05$). Close scrutiny of this scatter diagram reveals four units with extreme values of either twitch tension or change in surface representation amplitude which lie outside the main cluster of data points. It was felt that the correlation may have been unduly influenced by the inclusion of these strategically-placed values, however, when a correlation co-efficient was calculated for the same data without the values from these four units, the r value was in fact higher ($r = -0.77$). A second indicator of motor unit size is its force threshold of activation: Fig. 6.4*B* shows that there was also a significant negative correlation between the activation threshold of the motor unit and the change in its surface representation with time ($r = -0.55$, $P < 0.05$). There was no significant correlation between the contractile speed of the motor units and the change in the surface representation with time ($r = -0.17$, $P > 0.05$)(not shown).

It is a consequence of the size principle of recruitment of motor units (reviewed by Henneman and Mendell, 1981) that the total contractile force will be greater when a large unit fires at 10 Hz than when a smaller unit fires at 10 Hz. It may therefore be argued that the change in the surface representation may correlate not with motor unit size *per se* but rather with the total biting force during the contraction.

Several lines of evidence suggest that this is not the case. In most cases during the contraction the total biting force fell while the feedback unit was controlled at 10 Hz, so that by the 15th minute the force was usually less than 50% of its initial level. However, the change in the total force level could not be predicted from the initial biting force, and the units with the highest initial activation threshold were not necessarily those with the highest total force levels at the end of the contraction. In fact, there was no significant correlation between the total biting force in the last minute of the contraction and either the size of the motor

FIGURE 6.4

Relationship between the size of the motor unit and the change in amplitude of its surface representation after 15 minutes. The percent change in amplitude of the surface representation of the unit is plotted against *A*) the twitch tension of the unit (calculated from the first minute) and *B*) the recruitment threshold. The dashed lines are the linear regression lines of best fit. In *A* the correlation co-efficient (r) was −0.55 which was significant at the 5% level of confidence. In *B*, $r = -0.55$, which was also significant at the 5% level of confidence.

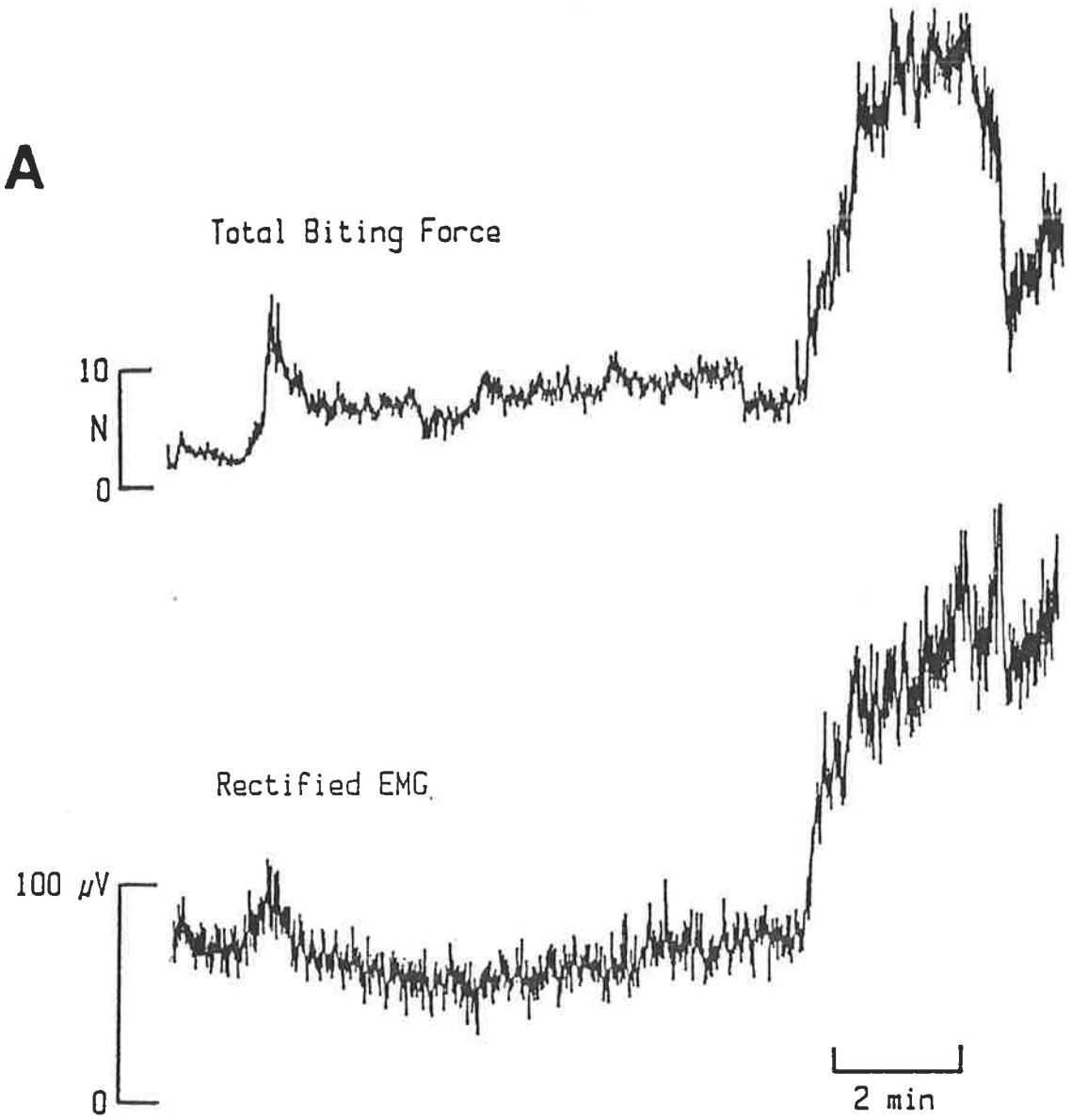

A
Change in Surface Representation Amplitude (%)
100
50
0
-50
0
50
100
Twitch Tension (x 10^{-2} N)
B
Change in Surface Representation Amplitude (%)
100
50
0
-50
0
20
40
60
Recruitment Threshold (N)

unit ($r = 0.44$, $P > 0.05$), the initial biting force ($r = 0.35$, $P > 0.05$), nor with the change in amplitude of the surface representation of the motor unit ($r = -0.27$, $P > 0.05$). These findings suggest that the total biting force did not influence the change in the unit's surface representation amplitude. In addition, the data from one unit in particular supports this conclusion. This unit had an initial activation threshold force of 2 N and an initial twitch force of 25 mN. The total biting force and rectified masseter EMG records are shown in Fig. 6.5*A* over the 15 minute period during which the unit's mean firing rate was controlled at 10 Hz by the subject. The biting force increased slowly over the first 12 minutes of activity, at which time there was a rapid, large increase in both the total biting force and the electrical activity in the masseter. The biting force peaked at 33 N at around 13 minutes and was still 21 N at 15 minutes. The average surface representation for the motor unit obtained from the first and 15th minute of the contraction is shown in Fig. 6.5*B*. Despite the fact that the total force level was the highest measured for any unit in the last 4 minutes of the contraction, there was little change in the surface representation amplitude over the 15 minutes. These data also provide an opportunity to assess the magnitude of errors in the averaged surface representation waveform postulated to occur as a result of motor unit synchrony. In this example, the total masseter EMG signal more than doubled between the first and 15th minute; the extra motor unit activity responsible for this must have increased the total error due to synchronization by a comparable amount in the trigger unit's surface representation (see Section 2.5). Despite this, this unit's surface representation amplitude was virtually unchanged, which suggests that the effect of motor unit synchronization on the surface representation is not large.

Further evidence regarding the effect of the total biting force was provided from those contractions where more than one motor unit was discriminated in the intramuscular records throughout the 15 minutes. When the surface representations of these background units were calculated the change in amplitude was not always in the same direction as that found in the unit controlled at 10 Hz. Fig. 6.6 shows one example where the surface contribution of two of three concurrently active single units (*A* & *B*) decreased by 16% and 9% respectively over the 15 minutes, while the contribution from a third unit (*C*) increased by 61% over the same period. The initial amplitudes of the three surface representations were: *A*, 16 μV; *B*, 8 μV; and *C*, 2 μV: *C* was barely detectable above the noise level in the first minute of the contraction. Since there is a positive correlation between motor unit size and the surface representation

FIGURE 6.5

The change in motor unit surface representation during a contraction in which the total biting force increased considerably. In *A*, the total biting force and the masseter smoothed rectified EMG are plotted against time during a continuous isometric contraction in which the mean firing rate of a selected masseter unit was controlled at 10 Hz throughout. The averaged surface representation waveforms for this unit, obtained from the 1st and 15th minute of the contraction are shown in *B*. Each average in *B* was the result of 512 counts.


A
Total Biting Force
10
N
0
Rectified EMG
100 μV
0
2 min

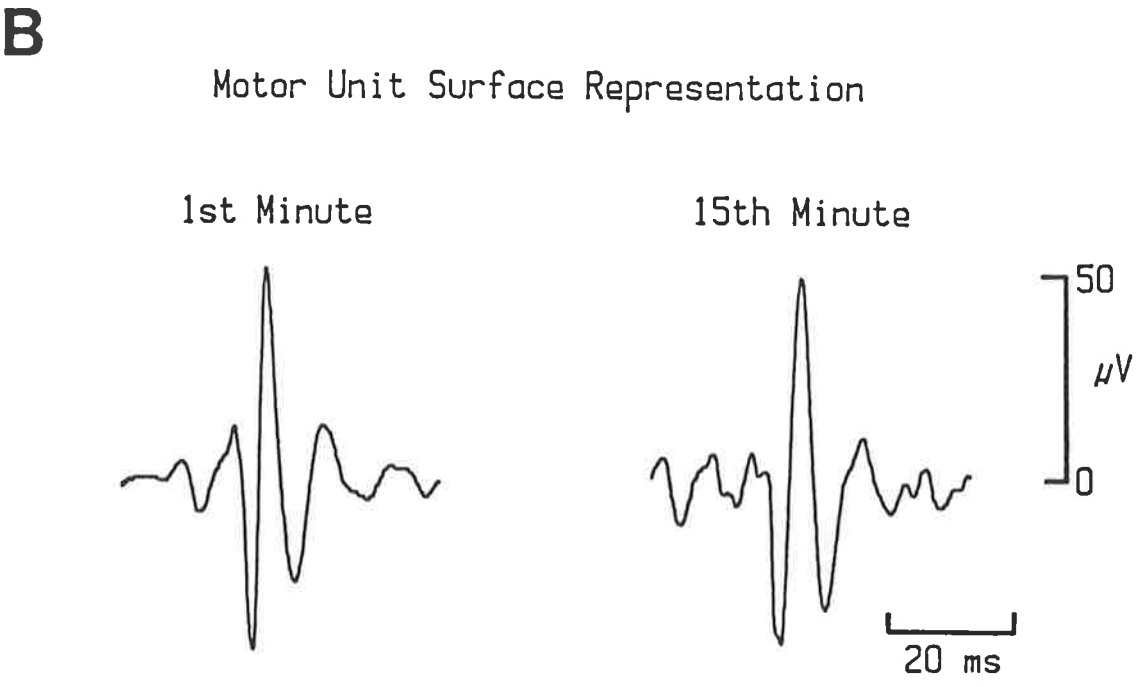
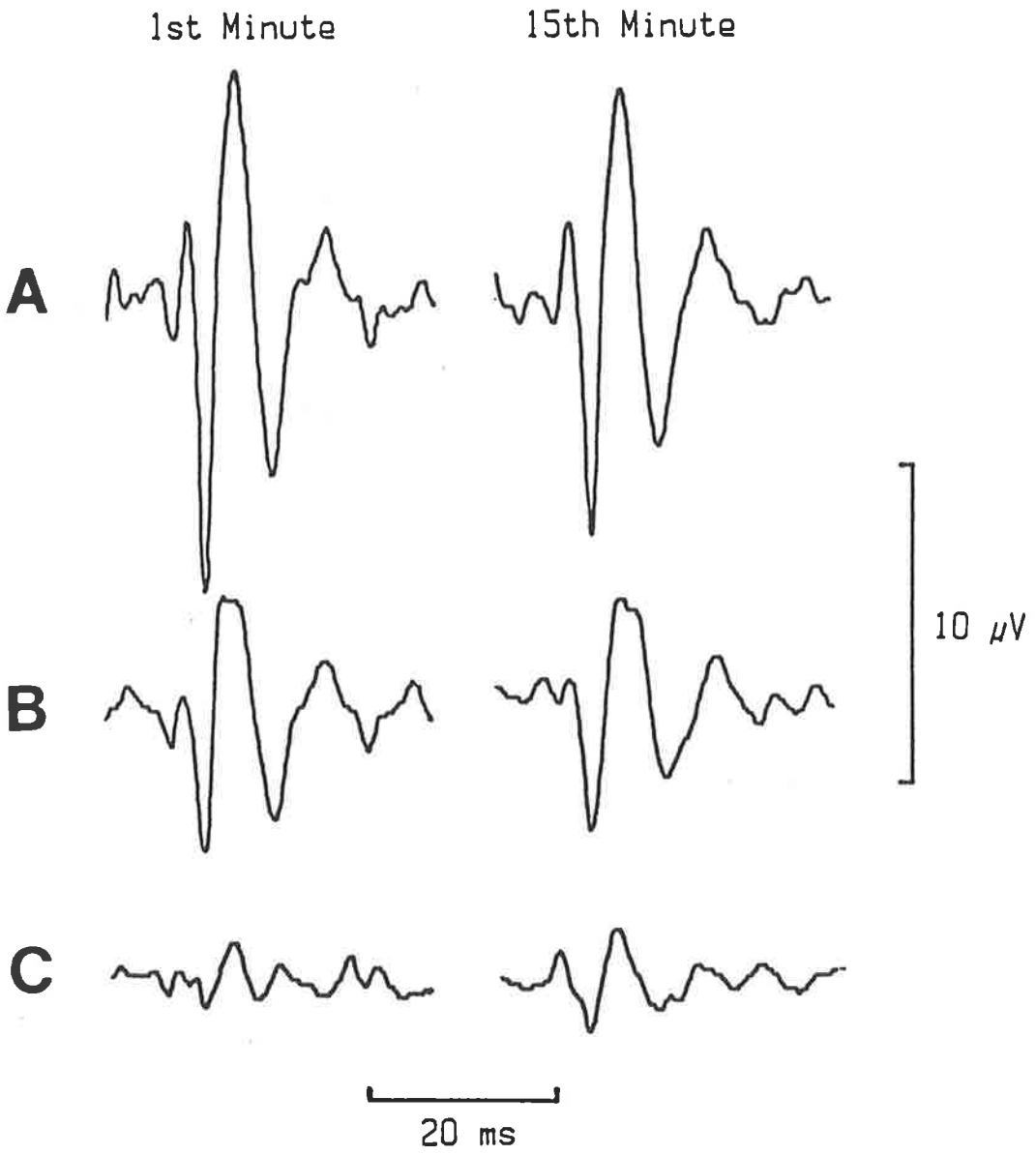

B
Motor Unit Surface Representation
1st Minute
15th Minute
50
μV
0
20 ms

FIGURE 6.6

Divergent changes in the amplitude of the surface representation of three concurrently – active units after 15 minutes of activity. On the left is the average surface representation waveform in the 1st minute and on the right the 15th minute. The amplitude of the surface representation of unit *A* (top trace) declined by 16% after 15 minutes. Similarly, the surface potential of unit *B* (middle trace) decreased in amplitude by 9% after 15 minutes. In contrast the amplitude of the surface representation of unit *C* (lower trace) increased by 61% over the same period. Each average was the result of 512 counts.


1st Minute
15th Minute
A
B
C
10 μV
20 ms

amplitude (Yemm, 1977*a*), it is likely that the relative sizes of the three units were $A > B > C$, in which case the changes in surface representation amplitude with time conform to the general pattern shown in Fig. 6.4 (i.e. small units increase, and larger units decrease surface representation amplitude with time). As the total biting force during the contraction was identical for all three units, the force level of the contraction could not be responsible for the differences among the units.

The changes in the unit's average action potential waveform (recorded intramuscularly) were not as consistent as those seen in the surface representation. In some cases the waveform shape changed quite dramatically over the 15 minutes, a result never seen in the surface representation. An example is given in Fig. 6.7. The upper traces are the averaged unit surface representation in the 1st and 15th minute of the contraction. The amplitude of the surface representation of this unit increased with time, but the waveform shape was otherwise relatively unchanged. The lower traces are the average intramuscular action potentials from the same epochs. In contrast to the stability of the upper trace, the waveform in the intramuscular record changed considerably with time. The pooled data from the 18 units revealed no significant correlation between the change in amplitude of the intramuscular motor unit potential and the change in amplitude of its surface representation with time ($r = -0.10$, $P > 0.05$).

6.4 Discussion.

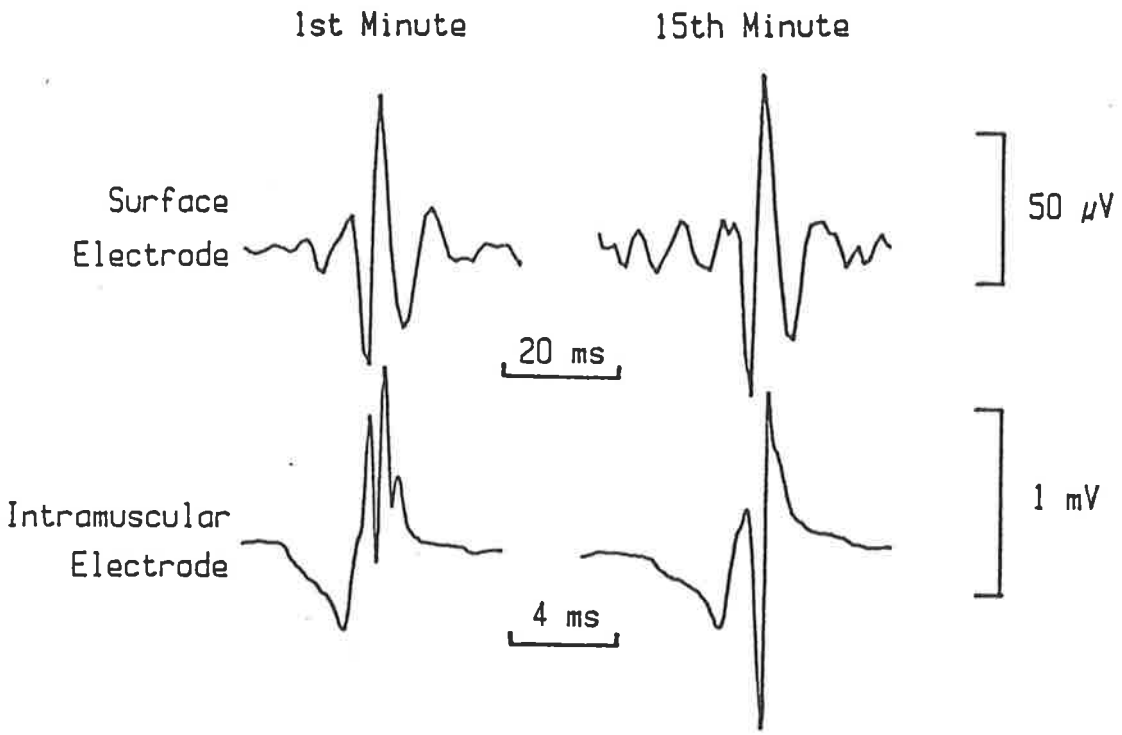
In the present study, under conditions of constant excitation, the contribution of a single motor unit to the surface EMG was not constant over 15 minutes. The change in amplitude of the motor unit surface potentials ranged from a 100% increase in one unit, to a 53% decrease in another, with most units showing some change in amplitude. In contrast, the duration of the single unit surface potentials was much more stable. I will first consider the possible explanations for these phenomena, before examining the relationship between the motor unit EMG signal and contractile fatigue.

General factors influencing the surface EMG signal.

The amplitude of any signal obtained by STA will be affected by the degree of synchronization of other motor units in the muscle with the triggering unit. The units included in the present study showed no evidence of correlated or synchronous activity using the rectified EMG technique of Milner-Brown *et al.*

FIGURE 6.7

A demonstration of the stability of the surface potential of the single unit compared to the action potential recorded with intra – muscular electrodes. The upper traces are the surface representations of a single unit potential calculated in the first (left trace, 603 counts) and 15th (right trace, 571 counts) minute of the contraction. There is a change in amplitude of the surface representation with time, but the shape of the waveform is otherwise essentially unchanged. Note that the duration of the waveform was unaffected. By comparison, the lower traces show the average intra – muscular action potential (64 counts) of this unit from the corresponding epochs, and a considerable alteration in the waveform is apparent.


1st Minute
15th Minute
Surface
Electrode
50 μV
20 ms
Intramuscular
Electrode
1 mV
4 ms

(1975) when tested at the start, or the end of the 15 – minute contraction. This was used as a gross estimate of the independence of motor unit discharge. In Chapter 2, it was shown by cross – correlation of unit firing times that most masseter units exhibit weak short – term synchronization with other units, and it was argued that this would introduce an error in the trigger unit's surface representation amplitude (Section 2.5). However, in the present study, the important parameter was the change in surface representation amplitude with time. In Chapter 2, no evidence was found for consistent time – dependent changes in the strength of synchrony during isometric contractions of the type performed in the present study. Therefore any synchronization – induced errors should substantially cancel out when the change in amplitude is the measured variable.

If there was a tendency for a consistent change in the number of active units, or their firing rates with time, this might influence the magnitude of the synchronization – induced error in unit surface representation amplitude. In general, the firing rate of the other active units remained relatively constant, but in approximately equal proportions of other concurrently – active units the mean firing rate increased or decreased over the 15 minutes (Chapter 9). Evidence of substantial recruitment of new units during this type of contraction was rarely seen in the intramuscular records. However, there were occasional exceptions which allowed an estimation of the likely effect of increased whole – muscle unit activity on the surface representation of a single unit. For several units, continuous activation at 10 Hz was accompanied by large increases in the total biting force and masseter EMG activity with time, which must have been produced by increased activity of other masseter units. This was the case for the unit shown in Fig. 6.5. Yet, despite the atypical large increase in activity of other units in the muscle, the amplitude of this unit's surface representation was virtually unchanged after 15 minutes. It seems likely that changes in whole – muscle excitation levels were not a significant source of error in the surface representation amplitude in the present study.

The amplitude of the EMG signal is also influenced by the distance between the active fibres and the recording site and their geometric relationship (De Luca, 1979). With surface electrodes these factors are essentially fixed and constant for the duration of the contraction. The jaw muscles have a number of advantages over limb muscles in this regard. The joint angle and muscle length are kept constant with high reliability in this experimental arrangement by the use of impressions of the incisor teeth on the bite bars which fix the relationship of the upper and lower jaws in all planes of movement. The masseter muscle is a fairly

short, wide muscle and firmly attached to bone over a wide area at each insertion without a tendon. This makes it less susceptible than most muscles to a contraction – induced distortion in shape. There will be some distortion of the muscle shape at high muscle forces, but in general the biting force during these experiments was not high.

The amplitude of the signal recorded by the surface electrodes may change if the resistance between the skin surface and the electrodes changes during the contraction (De Luca 1979). It is possible that perspiration or a change in the properties of the conductive gel used to improve the electrical contact could lead to a change in skin resistance. Two factors suggest that this is not significant. The first is the observed correlation between size of the motor unit and the change in the amplitude of the surface representation. Although a time – dependent change in the skin resistance cannot be ruled out, it is difficult to imagine that this could result in a systematic difference in the changes in the EMG of small and large single units. If anything, it would tend to weaken the correlation observed between size of the motor unit and the change in amplitude of the surface representation. Also, direct evidence was obtained on those occasions when additional units could be discriminated in the intramuscular record along with the unit controlled at 10 Hz by the subject. On most occasions the change in the surface representations of the units was similar; this was not surprising because the intramuscular electrodes were not selective enough to allow reliable discrimination of units with widely – differing recruitment thresholds during the same contraction because of superimposition of the action potentials from other active units. However, this was achieved on some occasions, and in these cases the changes in the units' surface representation amplitudes were quite different (e.g., Fig. 6.6). Clearly this type of divergent behaviour cannot be explained by a change in a non unit – specific property such as the skin resistance. It is also unlikely to occur as a result of a hypothetical synchronization – induced error due to a change in the whole – muscle unit activity, because this would be expected to have a similar influence on the surface representation amplitude of all units.

A change in the tissue filtering properties could influence the surface representation of the motor unit potential. Changes in the ionic balance of the extra – cellular fluid reported to accompany fatigue (Jones, 1981) could alter the filtering properties of the muscle. However, the two parameters that would be expected to correlate with this type of change; the total biting force and the fatigue index, did not correlate with the change in the surface representation.

Also, a change in the filtering properties of the muscle should induce a change in the duration of the waveform of the surface potential, but in general the duration was relatively constant, with the result that there was no correlation between the surface representation duration and amplitude changes ($r = 0.06$, $P > 0.05$). A change in the tissue filtering properties of the muscle also could not be responsible for the divergent changes in the surface representation of simultaneously active motor units such as those seen in Fig. 6.6.

It was hoped that the intramuscular records might help to explain the apparently unit-specific nature of the changes in surface representation of the motor unit potential because of the closer proximity of the recording electrode to the fibres. Unfortunately, the intramuscular records were not suitable for long-term comparisons of motor unit electrical activity (Fig. 6.7), presumably because of their sensitivity to minute changes in electrode/muscle fibre geometry (De Luca, 1979; also see Chapter 7). Units were only included in this study if the change in their intramuscular action potentials was observed to occur slowly and progressively with time, in order to be confident of the identity of the unit. Units with sudden changes in action potential waveform were excluded. Therefore the part of the action potential triggering the averager was relatively constant from trial to trial within each 1 minute epoch, and not a likely source of the variation in the averaged surface representation waveform. Comparison of the surface representation averages obtained from epochs in which the intramuscular action potential waveform had changed so that the averager was triggered by an earlier or later part of the waveform simply induced a shift in the surface representation average with respect to the trigger, but did not influence the shape of the surface representation waveform.

The unit-specific nature of the changes in the surface EMG

The evidence suggests that the change in amplitude of the surface representation of the unit potentials was the result of a change specific to the motor units themselves. The possible explanations for a reduction in motor unit EMG amplitude include:

i) Blocking of action potential propagation in some of the constituent fibres of the motor unit. The mechanisms include failure of neuromuscular transmission, failure of propagation at axonal branch points, and failure of propagation in the muscle fibre due to a raised threshold for excitation (Krnjevic & Miledi 1958).

ii) Reduction in amplitude of the action potentials of the constituent fibres (Krnjevic & Miledi 1958).

iii) Impairment of t – tubule membrane functions (Lännergren & Westerblad, 1987).

Blocking of action potential propagation.

There is disagreement regarding the existence of failure of neuromuscular transmission in human maximal voluntary contractions (Stephens & Taylor, 1972; Bigland – Ritchie *et al.*, 1979; Bigland – Ritchie *et al.*, 1982; Bellemare & Garzaniti, 1988). It is unlikely that failure of neuromuscular transmission would be a factor in the submaximal conditions of the present study; it is very secure at an activation rate of 10 Hz (Bigland – Ritchie *et al.*, 1979; Krnjevic & Miledi, 1958). It is possible for the nerve action potential to fail at axonal branch points (Krnjevic & Miledi 1958; Kugelberg & Lindegren, 1979), resulting in failure of propagation in some fibres of the motor unit. Although most evidence for axonal branch point failure has been obtained using high frequencies of stimulation (80 Hz or higher) there is evidence from animal studies that blocking of fibre action potentials is more likely under certain conditions such as hypoxia (Swadlow *et al.*, 1980) and accumulation of extracellular potassium (Smith 1980), and is also more likely to occur in larger motor units, perhaps because of the greater degree of axonal branching in these units (Parnas *et al.*, 1976; Spira *et al.*, 1976).

The evidence suggested that blocking of fibre action potentials was not responsible for the observed changes in motor unit EMG amplitude. Since the force output of the motor unit is proportional to the number of active fibres, blocking of fibre action potentials should correlate strongly with a reduction in twitch tension. However there was no correlation between the change in amplitude of the surface representation and the fatigue index in the present study (Fig. 6.3). It should also be possible to see direct evidence of fibre blocking in the intra – muscular records. When highly – discriminating, fine – wire, intramuscular electrodes are used it has been estimated that it is usually the potential from only one, or a few of the constituent fibres of the motor unit that is recorded (Buchtal & Schmalbruch, 1980). In this case, blocking of the action potential in one or all of the few fibres which contribute to the motor unit potential detected by the fine – wire electrode would result in either failure to detect a motor unit potential, or an altered potential which would not be recognized as belonging to the identified motor unit. The net result is that blocking of fibre action potentials should be accompanied by an increased standard deviation of the motor unit discharge as

fibre blocking proceeded. The variability of motor unit discharge during these contractions is studied in detail in Chapter 8. There was a general tendency for the variability of discharge to increase over the 15 minutes at a mean rate of 10 Hz, however there was no correlation between the change in variability and the change in the unit's surface representation amplitude (Fig. 8.4*A*). Therefore, blocking of fibre action potentials is unlikely to have been responsible for the change in amplitude of the motor unit surface potential.

Reduction of fibre action potential amplitude.

A second possible explanation for a change in the motor unit EMG amplitude is a reduction in the amplitude of the action potential of the constituent fibres of the motor unit. This can occur as a result of progressive depolarisation of the fibres (Krnjevic & Miledi 1958), or from a change in their ionic environment (Jones 1981). This type of change is accompanied by an increase in the waveform duration, since a decline in muscle membrane excitability is accompanied by a slowing of the conduction velocity, and the duration of the action potential recorded differentially with bipolar electrodes is inversely proportional to the conduction velocity of the muscle fibres (Lindström *et al.*, 1970). The stability of the duration of the surface potentials in the present study suggests that large changes in fibre propagation velocity did not occur. This finding would seem to exclude a reduction of muscle fibre excitability as the reason for the observed results.

Impairment of t-tubule membrane functions.

In a recent paper by Lännergren & Westerblad (1987), it was suggested that t-tubule conduction may be more vulnerable than the surface membrane to disruption after repetitive activation, and that impaired function in the t-tubule membrane could result in changes in shape of the conventionally recorded action potential. This remains a possibility for the observed results which deserves further study.

In summary, the explanation for the observed changes in single unit EMG is not immediately obvious. The correlation with motor unit size, and the divergent changes seen in some concurrently-active units suggests a unit-specific cause, rather than a non-specific cause, such as a change in the recording conditions. If the reduction in surface representation amplitude does reflect a change in the total electrical activity of the motor units, it is unlikely to have arisen from failure

of propagation of fibre action potentials, nor from a reduction in muscle fibre excitability, but the mechanism of the change remains unclear at present.

The correlation between motor unit size and the EMG changes.

In the present study, the motor unit property which best predicted the pattern of change in the surface representation amplitude was the size of the motor unit, estimated from either the twitch tension or the force threshold of activation. The larger the motor unit, the greater the decline in surface representation amplitude with time.

At first glance, this result is consistent with those obtained using stimulation of single units in animals, where many investigators have reported a decrease in motor unit EMG amplitude with prolonged activity. Most report that the fast motor unit population is more susceptible (Reinking *et al.*, 1975; Goslow *et al.*, 1977; McDonagh *et al.*, 1980; Clamann & Robinson 1985; Gardiner & Olha, 1987) while Wuerker *et al.* (1965) and Gardiner & Olha (1987) reported a correlation between the decline in the EMG and the motor unit size. In view of the strong correlation between speed and size of motor units in the muscles studied (usually cat or rat hindlimb) it is likely that a correlation between EMG amplitude reduction and size was present in all the above studies. The difference between these studies and the present study is that the stimulus rate was invariably considerably higher in the animal studies in which this effect has been noticed, with the likelihood that muscle activation failed due to neuromuscular transmission failure, or blocking of fibre action potentials. These possibilities do not appear likely in the present study.

EMG/fatigue relationship for single units.

The approach used in the present study allowed direct comparison of motor unit contractile fatigue and concomitant changes in its surface EMG signal. Therefore the ability of the surface EMG signal to reflect fatigue under these conditions could be directly assessed. Two properties of the surface EMG signal which have been used to infer fatigue are the amplitude, and the shift in the power spectrum of the signal towards low frequencies.

EMG amplitude and contractile fatigue.

The simultaneous measurement of single unit EMG and contractile fatigue during a voluntary contraction in humans has not been reported previously, although the two parameters are frequently measured together in animal single unit fatigue tests

as a means of assessing the degree of activation failure of the motor units. The poor correlation between changes in surface representation amplitude and contractile fatigue in the present study (Fig 6.3) suggests that muscle unit activation failure was not an important determinant of fatigue in this type of contraction.

A number of animal single unit studies employing high – frequency (>40 Hz) stimulation report a correlation between the change in amplitude of the single unit EMG and mechanical fatigue with continuous stimulation (Kugelberg & Lindegren 1979; McDonagh *et al.* 1980; Sandercock *et al.*, 1985; Clamann & Robinson, 1985). Experiments using a lower rate of stimulation more closely simulate activation patterns during prolonged voluntary contractions (De Luca, 1979; Bigland – Ritchie, 1981*b*). The fatigue resulting from this type of activation is believed to reside entirely within the contractile apparatus, and is probably due to a disturbance of excitation – contraction coupling (Edwards, Hill, *et al.*, 1977). It appears to be the most important type of contractile fatigue occurring in voluntary submaximal contractions, and can occur in all unit types with continuous 10 Hz stimulation, without a change in the single unit EMG waveform (Kugelberg & Edström, 1968; Sandercock *et al.*, 1985). In the present study, even though there were some changes in the single unit EMG amplitude with continuous low – frequency activation, there was a similar dissociation between the unit EMG and contractile fatigue. The poor correlation between the EMG signal and force is probably not surprising in view of the wide safety margin for excitation – contraction coupling. It has been stated by Falk (1961) that provided an action potential is of sufficient amplitude to propagate along the muscle fibre it is capable of fully activating the contractile apparatus.

These time – dependent changes in single unit surface EMG have implications for the use of the whole – muscle surface EMG in human submaximal fatigue studies. An increase in the amplitude of the total surface EMG signal (measured as either: integrated rectified EMG, smoothed rectified EMG, or the RMS amplitude) while the force is kept constant has been interpreted as indicating an increase in the excitation of the muscle to compensate for fatigue (e.g., Edwards & Lippold, 1956; Maton, 1981). It is apparent that attempts to infer a change in the number of active motor units and/or a change in their firing rate from the amplitude of the total surface EMG signal rely on the assumption that the contribution of the individual motor units to the surface EMG is invariant with time. Several recent reviews point out that in heavy, fatiguing contractions where the conduction velocity of the muscle changes, the contribution from each unit to

the amplitude of the total surface EMG signal will change because of the change in the duration of the potentials (Bigland – Ritchie, 1981*b*; De Luca, 1984). The present work shows that the contribution of a unit to the total surface EMG signal may change in amplitude even in low – force contractions where the duration of the potentials is unaffected. In view of the time – dependent changes in the contribution of individual units to the surface EMG signal found in the present study, it is concluded that comparison of the whole – muscle surface EMG signal amplitude at intervals during a long contraction gives only a very approximate estimate of the net excitation of the muscle.

EMG power spectrum and contractile fatigue.

The other property of the surface EMG signal which has been used to infer fatigue is its power spectrum. A shift in the power spectrum of the surface EMG signal towards low frequencies has been shown to accompany fatigue during forceful contractions (Kadefors *et al.*, 1968; Lindström *et al.*, 1970). The factors which may possibly affect the power spectrum of the surface EMG include: the firing rate and the degree of synchronous discharge of the individual units, time – dependent recruitment of new units with different spectral properties, and the increase in duration of the motor unit potentials resulting from a slowing of the conduction velocity. Most investigators consider that the principal cause of the large frequency shift in the surface EMG during forceful contractions is an increase in the duration of the motor unit action potentials (Lindström *et al.*, 1970; Mills 1982; Kranz *et al.*, 1983; De Luca 1984; Eberstein & Beattie, 1985).

In maximal contractions, where fatigue can be directly measured from the loss of force (constant excitation paradigm), there appears to be a good correlation between the shift to low frequencies in the power spectrum and contractile fatigue (Mills 1982). However, the nature of the relationship between the two is not clear, as during recovery the maximal force recovers quicker than the frequency shift in the surface EMG signal (Mills 1982), and the force from low frequency stimulation may be impaired for some time after the surface EMG signal has returned to normal (Moxham *et al.*, 1982).

The shift in the power spectrum of the surface EMG signal towards low frequencies has also been used to infer contractile fatigue during submaximal contractions (Häkkinen & Komi 1983), including Lindström & Hellsing (1983) and Naeije (1984) in the masseter muscle. The complex nature of the relationship between the EMG signal and force has led to some ambiguity when power spectrum changes are used to assess fatigue in submaximal contractions. The use

of a constant – force paradigm in this type of experiment makes direct measurement of fatigue in terms of loss of force impossible. The assumption is made that since the changes seen in the power spectrum of the surface EMG during moderately forceful submaximal contractions are similar to those occurring during maximal contractions, then contractile fatigue has occurred. This is probably true, but it is debatable whether contractile fatigue may be quantified from the EMG power spectrum alone, particularly in submaximal contractions, as has been suggested (Lindström *et al.*, 1977; Lindström & Hellsing, 1983; Palla & Ash, 1981). A direct cause and effect relationship has not been demonstrated between the loss of force and changes in the power spectrum of the surface EMG, and the dissociation between EMG power spectrum changes and force under certain conditions (e.g. Mills, 1982; Moxham *et al.*, 1983) make this approach unreliable.

Although the power spectrum of the surface EMG was not directly measured in the present study, the stability of the duration of the motor unit action potential suggests that large changes in the surface EMG power spectrum commonly attributed to fatigue did not occur during these experiments. Effects of time – dependent changes in synchronization or recruitment of new units on the EMG power spectrum are likely to be minor compared to the influence of action potential duration (muscle conduction velocity: Lindström *et al.*, 1970; Kranz *et al.*, 1983; Eberstein & Beattie, 1985), and in any event, motor unit synchronization did not change systematically during these experiments (Chapter 2), and recruitment of new units was rarely observed in the intramuscular records. The view that large shifts in the EMG power spectrum did not occur during the present experiments is supported by the study of Lindström & Hellsing (1983), in which incisor biting at forces below about 25 N (higher than the total force level at which most units were tested in the present study) did not produce a frequency shift in the masseter surface EMG power spectrum.

Despite the stability of the duration of the motor unit surface potential waveform, contractile fatigue was evident in a number of single motor units after 15 minutes of continuous activity at 10 Hz. These results suggest that power spectral analysis of the surface EMG signal is a poor indicator of contractile fatigue occurring during continuous, low – intensity contractions. This conclusion is in agreement with that of Sandercock *et al.* (1985), who measured EMG and contractile fatigue in cat hind – limb motor units during low – frequency stimulation (10 Hz) and found no correlation between the duration of the motor unit potentials and contractile fatigue, and also that of Moxham *et al.* (1983), in which low –

frequency fatigue was demonstrated in human muscle responding to electrical stimulation while the power spectrum of the surface EMG was normal.

Failure to recognize this has led to some misinterpretation of data. For example, several investigators have observed that there is a threshold level of sustained force below which a change in the power spectrum of the surface EMG does not occur (Kadefors *et al.*, 1968; Lindström and Hellsing, 1983). Lindström and Hellsing (1983), measured the changes in the EMG power spectrum in masseter muscle in man during sustained incisor biting at various submaximal force levels. They demonstrated that no changes occurred in the power spectrum of the EMG in all subjects with sustained biting below about 25 N total force. They interpreted this finding as indicating the total biting force below which no significant fatigue starts to develop. The demonstration of motor unit twitch fatigue in the present study (obtained under similar conditions) does not support this conclusion, and it should be emphasised that during low – intensity voluntary contractions, an absence of a change in the power spectrum of the surface EMG should not be interpreted as indicating that contractile fatigue has not occurred.

In conclusion, the time – dependent changes in amplitude of the single unit potential in the surface EMG signal under conditions of constant excitation suggest that the use of the surface EMG signal to infer constant excitation of the whole muscle is not reliable. Similarly, the assumption that an increase in amplitude of the total EMG signal during a constant – force, submaximal contraction indicates an increase in the excitation of the muscle to compensate for fatigue must also be treated with caution. In view of the demonstrated lack of a correlation between contractile fatigue and either the change in amplitude or duration of the single unit contribution to the surface EMG in the present study, it is apparent that some fatigue processes are not reflected in the surface EMG signal. These limitations should be considered whenever use of the surface EMG signal is contemplated to detect fatigue in submaximal, voluntary contractions.

CHAPTER 7.

LENGTH – RELATED CHANGES IN MOTOR UNIT ACTIVATION THRESHOLD AND ACTION POTENTIAL WAVEFORM.

7.1 Introduction.

The production of force by a skeletal muscle is a function of both the activity of the nervous system and the biochemical properties of the muscle fibres which it activates. The nervous system dictates which motor units are to contract and at what frequency, while the biochemical processes of the muscle fibres in each unit determine how quickly the fibres will contract, how much force they will produce, and how quickly they will fatigue. For most muscles, the nervous system recruits motor units in an orderly sequence so that, as the muscle force progressively increases, the first units to be activated are the small, slow twitch, fatigue – resistant motor units, while the large, faster – twitch, fatigue – susceptible units are recruited at higher muscle forces (reviewed in Burke, 1981*a*).

The direct evidence for this pattern of recruitment has been largely gathered by from animal studies. Clearly, however, there are many advantages in using human subjects for the investigation of motor unit behaviour since humans can be instructed to carry out precise and subtle voluntary motor tasks. It is, moreover, a simple matter to record motor unit potentials in human muscles.

The criterion which has been most widely used to characterize motor units in human studies is their isometric force threshold of activation. However, little attention has been paid to the factors which influence this important parameter. Büdingen & Freund (1976) have shown that the threshold depends on the rate of contraction of the muscle. In the present study, the force thresholds were found to depend on the muscle length. An incidental observation was that the waveform of the masseter unit potentials changed with the muscle's length, which may confound attempts to make inferences about the functional properties of individual motor units on the basis of the rise times and/or amplitudes of the unit potentials.

7.2 Methods.

Apparatus and general recording arrangements.

The details of the basic experimental arrangements have been given in full in Chapters 2 and 3. Motor unit action potentials were recorded with an intramuscular electrode inserted into the right masseter. The masseter surface EMG was also recorded. The subjects bit on stainless – steel bite bars with their incisor teeth, and biting force was measured by strain gauges mounted on the bars. The vertical separation of the bars could be altered smoothly and without backlash by turning a fine – pitch, worm – drive positioner. A similar positioner allowed antero – posterior alignment of the upper and lower bars to compensate for incisal overjet, and once set, remained fixed for each subject. In order to minimise the different mechanical advantages of different jaw openings, the bite bars were placed at an angle which bisected the vertical working range of the temporomandibular articulation.

Protocol.

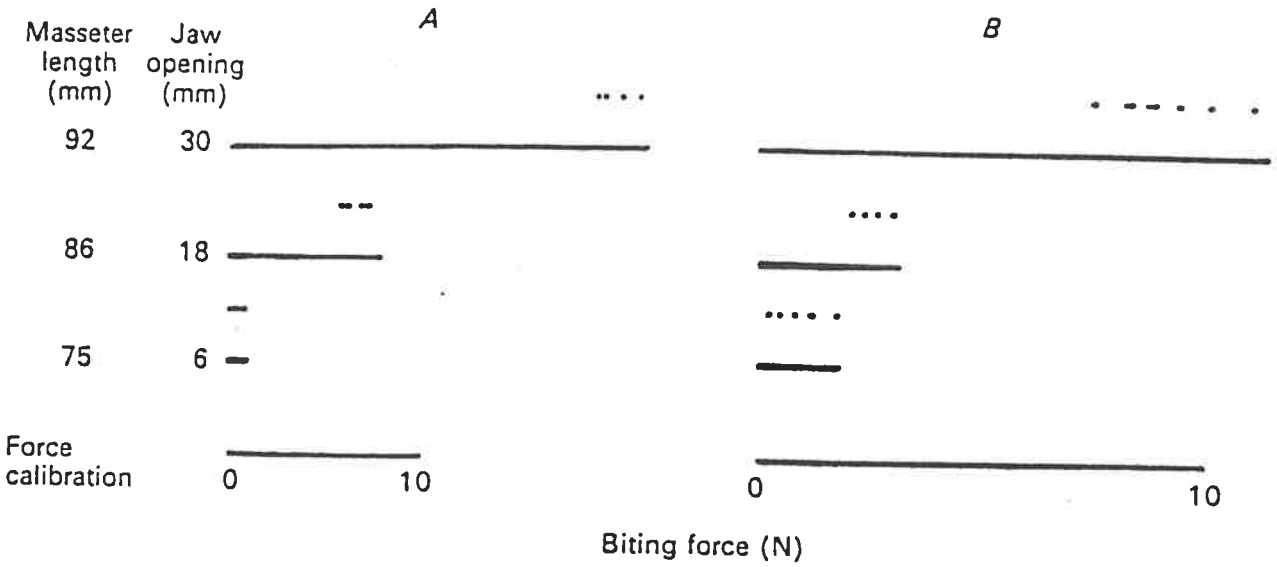
The subject was seated so that he/she could observe the motor unit potential (or standard pulses from an amplitude discriminator) on an oscilloscope screen. Auditory feedback was also provided. The subject was instructed to bite with sufficient force to make the unit fire continuously, but as slowly as possible. The usual result of this instruction was that the biting force fluctuated around the force threshold for the unit. This biting paradigm was repeated at 3 mm intervals between the minimum gape possible with the bite bars (6 mm inter – incisal distance) and the maximal gape that could be achieved by each subject. Whenever the inter – incisal distance was being changed, the subject ran the unit continuously so that the experimenters could be satisfied that the same unit was being observed at the different muscle lengths. Masseter length at each jaw separation was estimated by measurement of the distance between bony landmarks on the zygoma and mandible.

Analysis.

The force threshold was determined off – line by displaying the output of an amplitude discriminator set to give a standard pulse for each unit potential on the vertical axis of a storage oscilloscope against the biting force on the horizontal axis. The data for two motor units at three different muscle lengths are shown in Fig. 7.1. As the biting force increased at each muscle length, the oscilloscope

FIGURE 7.1

Method for determining threshold activation force for each motor unit: data from two units (*A* & *B*) at three muscle lengths in the same subject. Photograph of storage oscilloscope displays in which horizontal displacement of the beam was produced by the output of the biting force transducer, and each dot represents a motor unit action potential (the dots are the output from an amplitude discriminator). The activation threshold is the lowest force at which the unit fired at that muscle length. The horizontal line below the dots at each muscle length indicates the range of biting force over which the unit was tested at that muscle length, i.e. as the biting force fluctuated around the activation threshold. For the purpose of clarity, only a few of the hundreds of dots recorded at each muscle length are shown. The horizontal line at the bottom of the screen is the force calibration signal of 10 N for both units.


A
B
Masseter length (mm)
Jaw opening (mm)
92
30
86
18
75
6
Force calibration
0
10
0
10
Biting force (N)

beam traced a horizontal line at ground potential from left to right on the screen. When the force reached the threshold for the unit, the discriminated unit pulse registered as a dot on the screen at that force level. The movement of the beam along the line below the unit dots indicated that biting force fluctuated almost continuously through the firing threshold. The operational definition for force threshold at each muscle length was the lowest biting force at which the unit fired; i.e., the left – most dot at each level of the display in Fig. 7.1*A* & *B*. (For unit *A* in Fig. 7.1, the thresholds were 0.1 N at 75 mm; 6 N at 86 mm and 18 N at 92 mm.) Occasionally, subjects gave an accidental, rapid bite which produced a dot far to the left of the cluster of dots around the operationally – defined threshold. These values were readily identified and rejected.

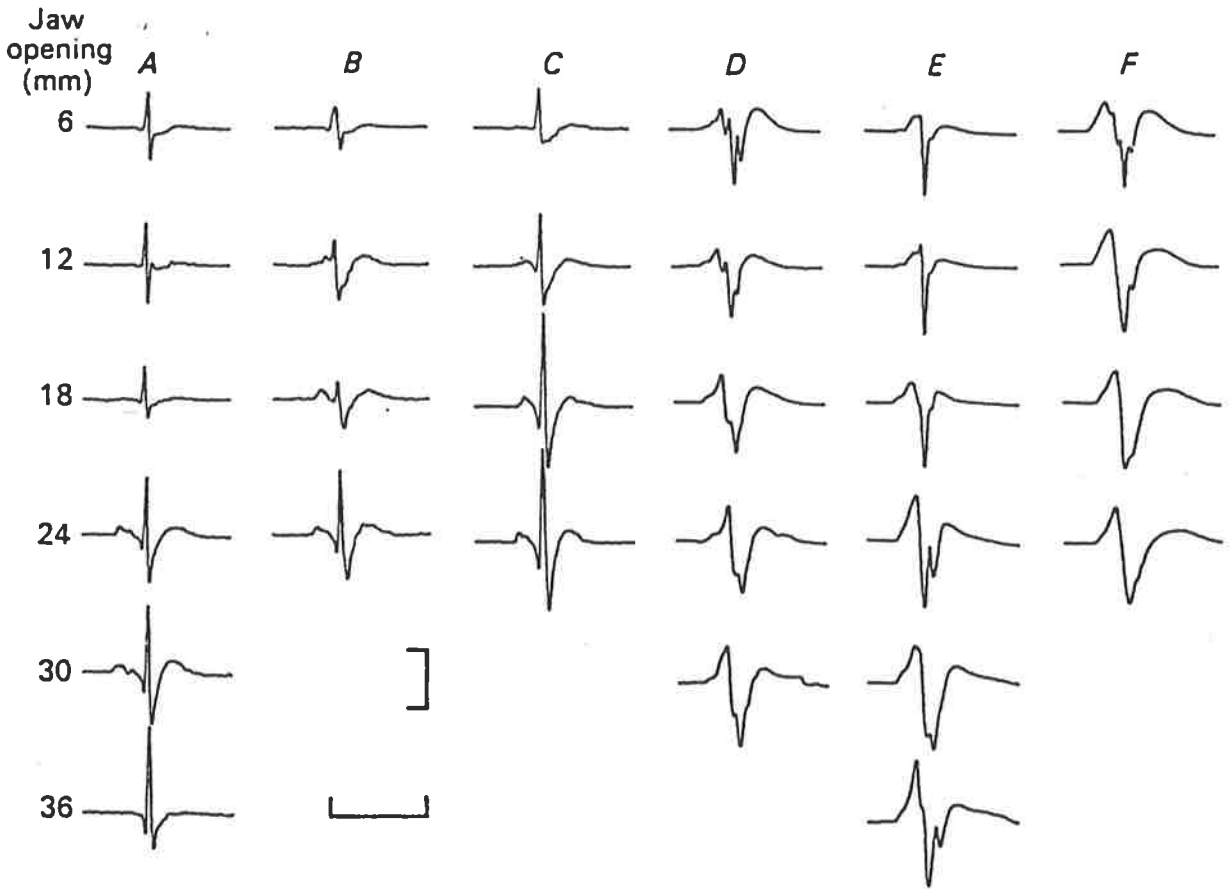
Data from several units over a wide range of muscle lengths were acquired from each of three subjects. For these subjects, an additional experiment was carried out several weeks later to determine approximately the length – passive tension characteristics of the jaw – closing musculature and related soft tissues. Surface electrodes were placed on the temporalis, masseter and digastric muscles. The subject then positioned his teeth on the bite bars and, with the aid of visual feedback, attempted to keep the surface electromyogram (EMG) activity of these muscles at the resting level. The force exerted on the bite bars was then measured as the inter – incisal distance was increased passively in 3 mm increments from 6 mm to maximum comfortable gape, then back again. The maximal voluntary biting force at 6 mm opening was also determined for two of these subjects.

7.3 Results.

During the passively applied changes in muscle length, the shape and amplitude of the unit potential was observed to change in virtually all units. The possibility that the apparent change in shape was due to substitution of one unit for another was ruled out on the grounds that the shape change always occurred progressively as the jaw separation was slowly changed (approximately 0.2 mm/s). Examples of six unit potentials recorded at different muscle lengths are given in Fig. 7.2. In each unit, the amplitude, rise time and shape of the waveform changes gradually but dramatically over the working length of the muscle. In particular, singularities in the waveform became more or less prominent as the length changed, rather as if the filtering characteristics of the recording amplifier had been changed. The changes in waveforms with length were reversible, with the unit potentials

FIGURE 7.2

Change in wave form of masseter motor unit potentials at different jaw openings (muscle lengths). The wave forms of six different units are shown as the jaw was opened in 6 mm increments: each wave form shown was obtained by averaging sixteen action potentials. The effective bandwidth of the recording system for this procedure was $20-2500$ Hz. The amplitude calibration is 50 μV for units $A-C$ which were recorded from one subject, and 500 μV for units $D-F$ which were recorded from a different subject. The time calibration is 5 ms.


Jaw opening (mm)
A
B
C
D
E
F
6
12
18
24
30
36

reverting progressively to original shape as the jaw separation was returned to the 6 mm inter – incisal separation. No discernible pattern in the change in waveform with length was observed from one subject to another.

The force thresholds for units recorded at the 6 mm inter – incisal positions were between 0.1 N and 49 N, measured at the incisor teeth. The method used for force threshold determination at a given muscle length was reproducible. Fig. 7.3*A* shows the results of an experiment in which the measurements of isometric force threshold of a single unit over the functional muscle length were repeated on two consecutive opening trials.

As the jaw separation increased, the thresholds of all units progressively increased. Fig. 7.3 shows the threshold forces (open squares) for four units in subject *B*, and three units in subjects *C* and *D*, plotted against masseter length. In some units the thresholds were measured as the jaw was progressively opened, and in others as it was closed, as indicated by the arrows on the continuous lines.

The dashed lines superimposed on the threshold data for subjects *B*, *C*, and *D* in Fig. 7.3 are the length – passive tension curves for the combined jaw musculature and related soft tissues which were measured some weeks after the unit recordings were made. The atypical inflection in the passive tension curve for subject *B* near the position of maximal muscle length appeared to correlate with a "click" in this subject's right temporomandibular joint. The maximal voluntary incising force at 6 mm jaw opening was 200 N for subject *B* and 150 N for subject *D*.

Threshold values over the whole range of muscle lengths were obtained for comparatively few units, owing to the difficulty of continuing to record from the same unit when the muscle was stretched. One example of a unit in which complete data were obtained is shown in Fig. 7.4. In this instance, threshold values were obtained first as the jaw was opened, then as it was closed (continuous lines with arrows). The hysteresis between the threshold values obtained during opening and closing is similar to that in the passive tension curve of the jaw musculature in this subject.

7.4 Discussion.

The identification of a single motor unit potential is a subjective process in which the observer depends on the constancy of various features of the waveform of the action potential. These features include the amplitude, rise time, polarity and any singularities of shape that may be present. In the present study, some or all of

FIGURE 7.3

Activation thresholds for masseter motor units and passive jaw – closing forces at different muscle lengths. Open squares are threshold values for various single units at different muscle lengths: filled circles are passive jaw – closing forces recorded at various muscle lengths during progressive jaw opening, then closing. *A*: threshold forces for a single unit recorded during two separate cycles of progressive jaw opening, showing that the method used for threshold determination is reproducible. *B*: data from one subject showing threshold forces for four units at various muscle lengths, and the passive tension recorded during opening then closing (opening and closing indicated by arrows). *C*, *D*: data from two other subjects arranged as in *B* (three units in each subject).

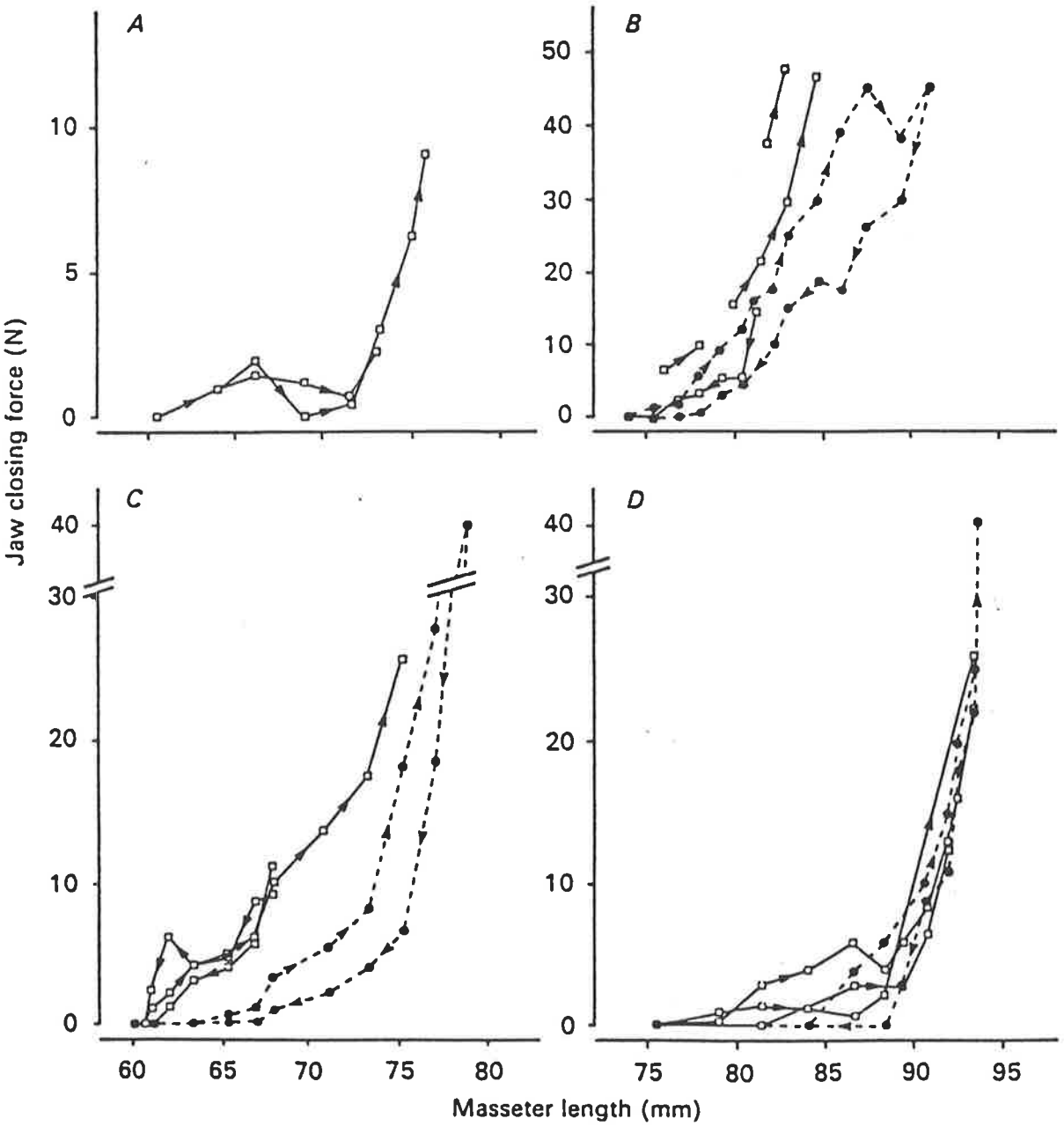
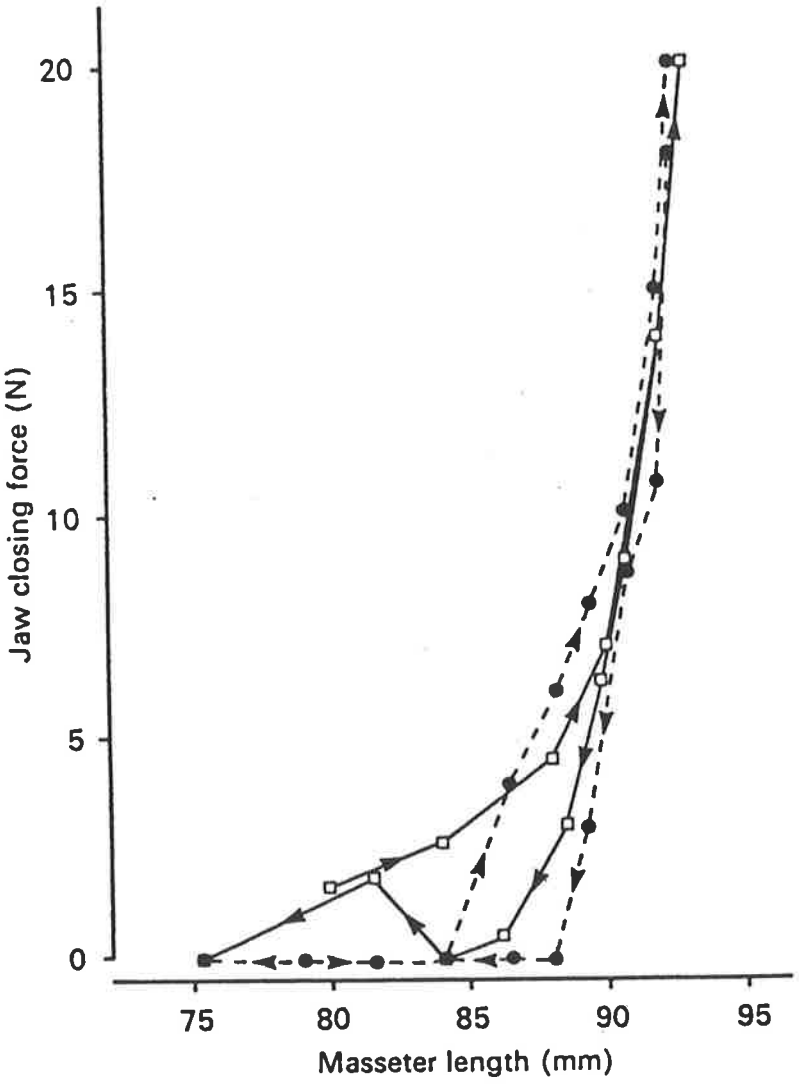

A
B
C
D
Jaw closing force (N)
Masseter length (mm)
0
5
10
20
30
40
50
60
65
70
75
80
85
90
95

FIGURE 7.4

Passive tension of the jaw – closing apparatus superimposed on the activation thresholds for a single motor unit at various muscle lengths during progressive jaw – opening, then closing. The open squares are the activation thresholds for this unit recorded as the masseter is first lengthened in increments (jaw – opening), then shortened. The filled circles (joined by dashed lines) indicate the passive jaw – closing force recorded at various muscle lengths as the jaw was opened then closed step by step. A similar pattern of hysteresis between opening and closing is evident in both the unit force threshold data points and passive tension.


20
15
10
5
0
Jaw closing force (N)
75
80
85
90
95
Masseter length (mm)

these features of each unit potential varied when the muscle length was changed. This is most likely to be due to the alteration in the geometric relationship of the unit's muscle fibres to the bipolar recording electrode, although the presumed change in muscle conduction velocity with length may also be significant.

This observation has implications for several experimental situations. For example, it would clearly be difficult to be confident of the identity of a single motor unit in a muscle which was shortening rapidly if its waveform were to change like those shown in Fig. 7.2. In other studies, attempts have been made to correlate various features of the unit potential waveform and with the functional characteristics of the unit (e.g. Olson *et al.*, 1968; Buchtal *et al.*, 1973; Tanji & Kato, 1973*a*; Goldberg & Derfler, 1977). The relatively poor correlations seen in these studies are not surprising in the light of the present observation that the waveform of a motor unit can vary dramatically with muscle length. The dependence of waveform on the geometric relationship between the electrode, the motor unit being studied and the whole muscle has been discussed by De Luca (1979).

The most widely used criterion for describing motor units in man is the total muscle force at which the unit is recruited. The ramp technique gave rather inconsistent values for absolute force threshold in successive biting trials. It is known that, at a given muscle length, the activation force threshold of a unit depends strongly on the rate of isometric contraction (reviewed in Freund, 1983). Hence, the conventional method for determining force threshold is to use controlled rates of increase of force. However, the increase in isometric force produced by voluntary effort is never a completely smooth ramp. Hence, it may well be that the small, rapid fluctuations superimposed on the force ramp are significant determinants of the force threshold with this method. In any event, standardisation of the rate of increase of isometric force will not necessarily produce the same rate of change of sarcomere length at one muscle length as it will at another, because of the length – tension and force – velocity relationships of skeletal muscles (Hill, 1938). A new operational definition for force threshold was therefore chosen in the present study, *viz.*, the lowest value of biting force at which the unit fired while the force changed slowly and randomly. This method gave quite reproducible results from one trial to another (Fig. 7.3*A*), and appears to offer some advantages over the ramp technique, particularly when the muscle length is to be changed.

The criterion of recruitment threshold is widely used to describe motor units not only because it is easy to measure, but also because it is usually considered that this parameter gives some insight into the functional characteristics of that unit. It is often assumed in human studies that the motor units activated at low forces have slow, low – amplitude twitches and are resistant to fatigue, while those recruited at higher forces are likely to be more powerful, fast – twitch units which are more susceptible to fatigue (reviewed in Burke, 1981*a*; Freund, 1983). The present observations provide a cautionary note on the general applicability of this assumption, since the threshold for activation of some units varied consistently by as much as an order of magnitude when the muscle length was changed (e.g. Figs. 7.3 & 7.4). This problem becomes more pronounced if unit activation thresholds are described in terms of a percentage of the force of maximal voluntary contraction (MVC) at various muscle lengths. The maximal incising force decreases by about 30% when the jaw position is changed from 15 mm to 30 mm inter – incisal distance (Mackenna & Türker, 1983). For the units shown in Fig. 7.3*D*, for example, the threshold forces would be about 1% MVC at the resting muscle length of about 83 mm (equal to about 15 mm inter – incisal distance) and 17% MVC at 95 mm (30 mm inter – incisal). At least in the jaw muscles, therefore, force threshold alone is an inadequate criterion for assigning individual motor units to one of the accepted categories of motor unit types.

The data presented suggest that the change in activation threshold with length is secondary to the length – tension relationship of skeletal muscles. It is clear from Figs. 7.3*B* – *D* and 7.4 that the activation thresholds of masseter units closely follow the passive tension curves of the jaw musculature over the functional range of jaw openings. To appreciate why this may be so, it is necessary to consider the basis for the activation thresholds for any particular motor unit. If a given motor command signal is just sufficient to activate a particular motor unit, then the threshold force for that unit is the force produced by all the units of lower thresholds firing at frequencies determined by that level of motor command signal. If this motor command signal is kept constant while the muscle length is changed (without feedback) these units will continue to fire at the same frequencies, but their force output will change due to the length – tension curve of that motor unit population (Gordon *et al.*, 1966). In the case of masseter motor units, the passive tension of the jaw musculature increased sharply from about the mid – open position onwards. Thus, the threshold forces of the units would also be expected to rise sharply over this range: this is what was observed. This explanation is given additional support from the data shown in Fig. 7.4, in which the pattern of

opening – closing hysteresis in the passive jaw – closing force was reflected closely in the unit activation thresholds. The passive jaw – closing forces recorded in these experiments were considerably larger than those reported by Lynn & Yemm (1971). This may be because (a) their protocol did not include feedback of jaw muscle EMG signals to reduce the likelihood of a contribution from muscle contraction, and (b) the extent of the jaw – opening movement appeared to be less in their subjects than in the present study.

Other hypotheses could be advanced to explain the length – dependent changes in motor unit threshold. For example, sensory feedback could act in several different ways to modify the excitability of motoneurones at different muscle lengths. This feedback could conceivably alter the recruitment order of motoneurones at different muscle lengths so that units which are recruited at low forces when the muscle length is short are not recruited until higher forces are reached at longer muscle lengths. However, no units were found in which the threshold was low at a wide – open position then subsequently became higher when the teeth were nearly together, as would be expected if major shifts in recruitment order had taken place. It is therefore contended that the sharp increase in threshold for all units which occurs near the maximal muscle length and the similarity in the pattern of opening – closing hysteresis in both the length – passive tension curves for some units argue strongly in favour of the explanation that the change in activation threshold with length is a consequence of the length – tension properties of skeletal muscle. The extent to which these findings are applicable to other muscles will depend on the active and passive length – tension curves of the muscle concerned and the mechanics of the joint about which that muscle is acting.

CHAPTER 8.

OBSERVATIONS ON THE VARIABILITY OF MOTOR UNIT DISCHARGE IN THE HUMAN MASSETER.

8.1 Introduction.

There is a body of evidence, derived mainly from studies in the cat hindlimb, which indicates that the different types of motor units have differences in their motoneurone properties (reviewed by Burke, 1981*a*; Stuart & Enoka, 1984). Anatomical differences in cell size and axon diameter are found for type S and type F motor units. Differences in motoneurone biophysical properties (e.g. input resistance, rheobase, afterhyperpolarization potential (AHP)) are also found for units of different type. Some of these differences should be reflected in the repetitive discharge properties of motoneurones belonging to different motor unit types. For example, motoneurones of type S motor units show less adaptation in firing rate to a constant – current injection than motoneurones of type F motor units (Kernell & Monster, 1982*b*), and are thus much better suited for tonic contractions. Differences in AHP have also been suggested to influence motoneurone firing rate and regularity (Person & Kudina, 1972; Derfler & Goldberg, 1977).

Correlations between normal firing patterns and motor unit physiological properties are difficult to study in animals for technical reasons. Motoneurone firing patterns are different in the intact cat during locomotion compared with reduced preparations (Hoffer *et al.*, 1987), which are necessary for conventional motor unit typing in animals.

On the other hand, normal motor unit firing patterns are studied easily in humans during voluntary contractions. Tokizane & Shimazu (1964) measured ISI variability in a number of different muscles and proposed that units in all muscles could be subdivided into two general categories: a "tonic" pattern with low firing rates and regular discharge, and a "kinetic" pattern with higher discharge rates and more irregular discharge patterns. Subsequent studies have generally failed to find a clear division of motor units into these groups, but suggest a more continuous range of firing properties between these extremes (Clamann, 1970; Person &

Kudina, 1972; Stålberg & Thiele, 1973; Tanji & Kato, 1973*b*; Hannerz, 1974; Freund *et al.*, 1975).

There have been several attempts to correlate motor unit firing patterns with other physiological properties of motor units in humans. Warmolts & Engel (1972) studied patients with motor neurone disease using open – biopsy electromyography, and found that motor units capable of sustained firing with regular discharge patterns were present in the muscles with a preponderance of type I fibres on biopsy. Conversely, motor units from muscles containing mainly type II fibres fired irregularly in bursts, and could be activated only during rapid, vigourous contraction.

Freund *et al.* (1975) found no consistent differences in the ISI variability of high – and low – threshold motor units in human FDI. Hannerz (1974) studied motor units in the human anterior tibialis muscle and found that all units with low recruitment thresholds (less than 30% MVC) discharged tonically, while units with high recruitment thresholds (>80% MVC) could only fire intermittently. Units with a lower recruitment threshold fired at lower rates with less variability than higher threshold units. In a later study in the short toe extensors, a correlation was found between motor unit firing properties and axonal conduction velocity (Borg *et al.*, 1978). Motor units with low axonal conduction velocity were of the tonic discharging type. It is likely that the high – threshold units fire intermittently because of a rapid adaptation of the parent motoneurone to input currents (Grimby *et al.*, 1981), and thus are not capable of true tonic discharge during normal voluntary activation.

These earlier studies have correlated motor unit discharge properties with recruitment threshold and axonal conduction velocity, but only general inferences can be drawn from these measurements regarding the physiological type of the units encountered. In this Chapter, the variability of motor unit discharge in the masseter is analysed, and correlations sought between this variable and the physiological properties (size, contractile speed and fatiguability) of the units which were described in Chapter 5.

8.2 Methods.

Apparatus and recording procedure.

These arrangements were identical to those previously described in detail in Chapter 2.

Protocol.

This was also identical to that described in Chapter 5. The subject controlled the mean firing rate of a selected unit at 10 Hz for 15 minutes during a continuous isometric contraction with the aid of audio and visual feedback. The unit controlled by the subject will be referred to hereinafter as the "feedback" unit. In most cases the feedback unit was one of the units whose fatigue data were presented in Chapter 5.

Analysis.

The intramuscular EMG records were analysed off-line to determine the firing pattern of the motor units recorded during the contraction. Motor unit action potentials in each record were discriminated using the SPS 8701. The interspike intervals (ISI's) for each discriminated unit were stored on disk. Only individual spike trains that could be discriminated with high reliability (less than 1% recognition errors) were used for the analyses presented in this Chapter. In experiments in which only one unit was detected by an electrode, this degree of discrimination accuracy was always achievable. On the occasions in which action potentials from more than one unit were detected on an electrode, the feedback unit could usually be discriminated with the required accuracy because it was commonly much larger in amplitude than the background units (thus minimising the number of recognition errors resulting from superimpositions).

The primary goal was to obtain an estimate of the variability of motor unit discharge for the units controlled by the subject at a mean rate of 10 Hz with the aid of feedback. This can be obtained by measuring the standard deviation (SD) of a series of ISI's over a period of time. It is known that the SD increases as the mean ISI increases in human motor units during a voluntary contraction (Tokizane & Shimazu, 1964; Derfler & Goldberg, 1977). Therefore, in order to make comparisons of ISI variability, each SD value must apply to a fixed value for mean ISI. The subjects were instructed to control the mean ISI of the feedback unit at 100 ms, but were not able to control their motor units so precisely that the mean ISI was exactly 100 ms in a particular epoch. Therefore it was necessary to estimate the value of the SD for an ISI of 100 ms for each unit. This was achieved in the following manner (after Eriksson *et al.*, 1984):

The mean ISI and SD was calculated for consecutive 10-second epochs during the initial 2 minutes of the continuous isometric contraction, using a built-in function of the SPS 8701. This resulted in 12 values of mean ISI and SD in

this 2-minute epoch, which were plotted (e.g., closed circles in Fig. 8.1), and a linear regression performed. The equation of the linear regression line-of-best-fit was used to estimate the SD at an ISI of 100 ms for the unit. The estimate of SD obtained in this manner for values from the initial two minutes of the contraction was designated SD_S. In Fig. 8.1 this is indicated by the dashed line intersecting the y axis at 28.24. In order to detect any change in the unit's discharge variability following prolonged activation, the same procedure was followed for 10-second epochs during the last 2 minutes of the contraction (minutes 14 & 15) (open circles in Fig. 8.1). The linear regression equation fitting these points was used to estimate the SD at an ISI of 100 ms for the last 2 minutes of the contraction. This value was designated SD_e (in the example given in Fig. 8.1 it was 36.77). This process was repeated for each feedback unit. The values SD_S and SD_e are reliable estimates of the SD at a mean ISI of 100 ms provided that 100 ms is within the range of mean ISI values used in the linear regression calculation. This was always the case for the data included in the analysis.

The values SD_S and SD_e from all units were grouped, and tested for significant differences using one-way ANOVA. For each unit, an index of the change in interval variability with time (SD_{ch}) was calculated according to the formula: $SD_{ch} = SD_e/SD_S$.

It was frequently possible to detect activity of other motor units in addition to the feedback unit on the same, or separate electrodes during the contraction. The firing-rate behaviour of these "background" units is analysed in detail in Chapter 9. These units displayed a range of mean firing rates, usually above 10 Hz. Where possible, the action potentials of these units were discriminated and the intervals stored. The mean ISI and SD for 10-second epochs from the first two minutes of the contraction were calculated for each background unit. The data from a number of units firing at different mean rates in the same subject were pooled in order to give an indication of the effect of the mean ISI on the SD.

8.3 Results.

Thirty seven motor units from 10 subjects were controlled at a mean rate of 10 Hz for 15 minutes, and used to obtain values of both SD_S and SD_e as shown in Fig. 8.1. The extent of the change in discharge variability with time for the individual units is seen more clearly in Fig. 8.2, which is a plot of the initial discharge variability (SD_S) vs. the index of the change in discharge variability over

FIGURE 8.1

The method for estimation of the standard deviation of a unit's discharge at a mean ISI of 100 ms, and the change in this value with time. The values of mean ISI for a single unit are plotted against the SD for 10-second epochs from the initial 2 minutes (filled circles) and final 2 minutes (open circles) of continuous activation. Regression lines are fitted to each group of data points. The regression equations were used to calculate the SD at a mean ISI of 100 ms for each group of points; SD_s from the initial 2-minute epoch and SD_e from the final 2-minute epoch. In the examples shown, the regression line used to calculate SD_s had a correlation coefficient (r) of 0.83, and the line used to calculate SD_e had an r value of 0.86.

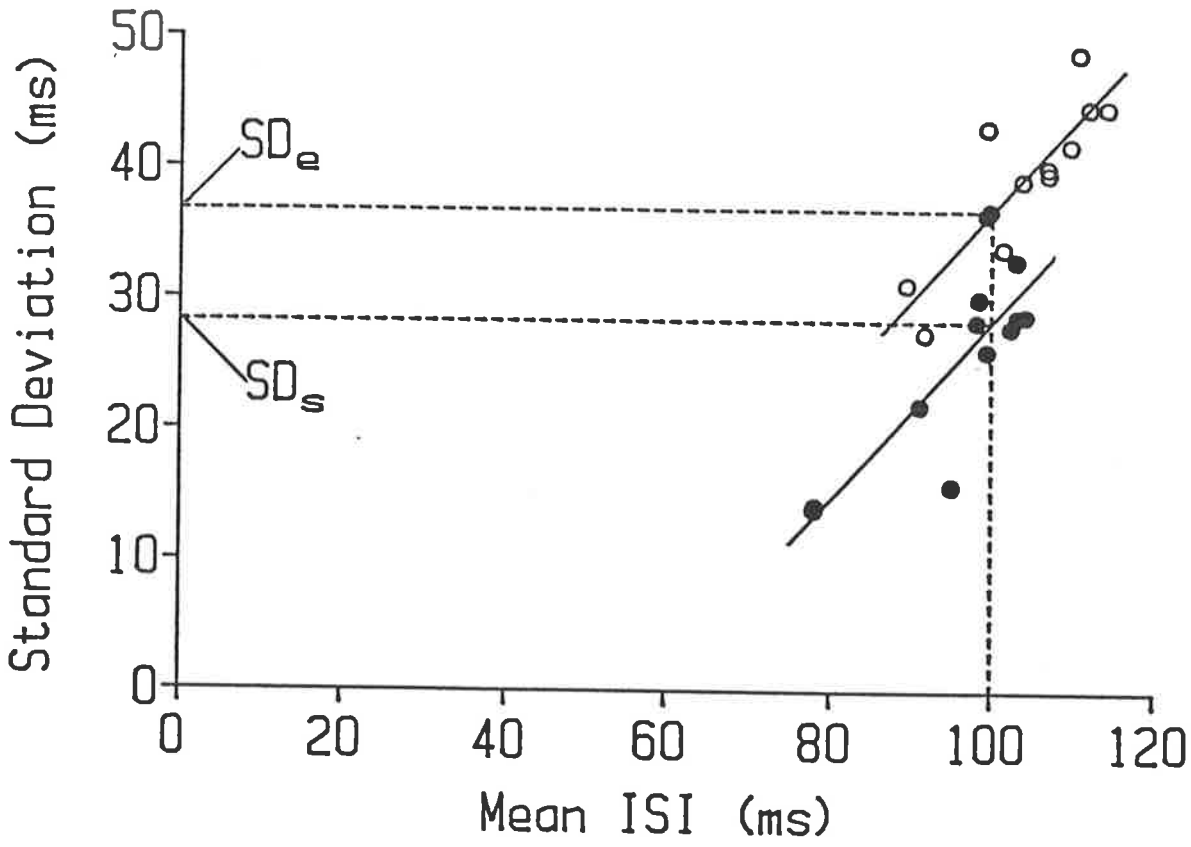
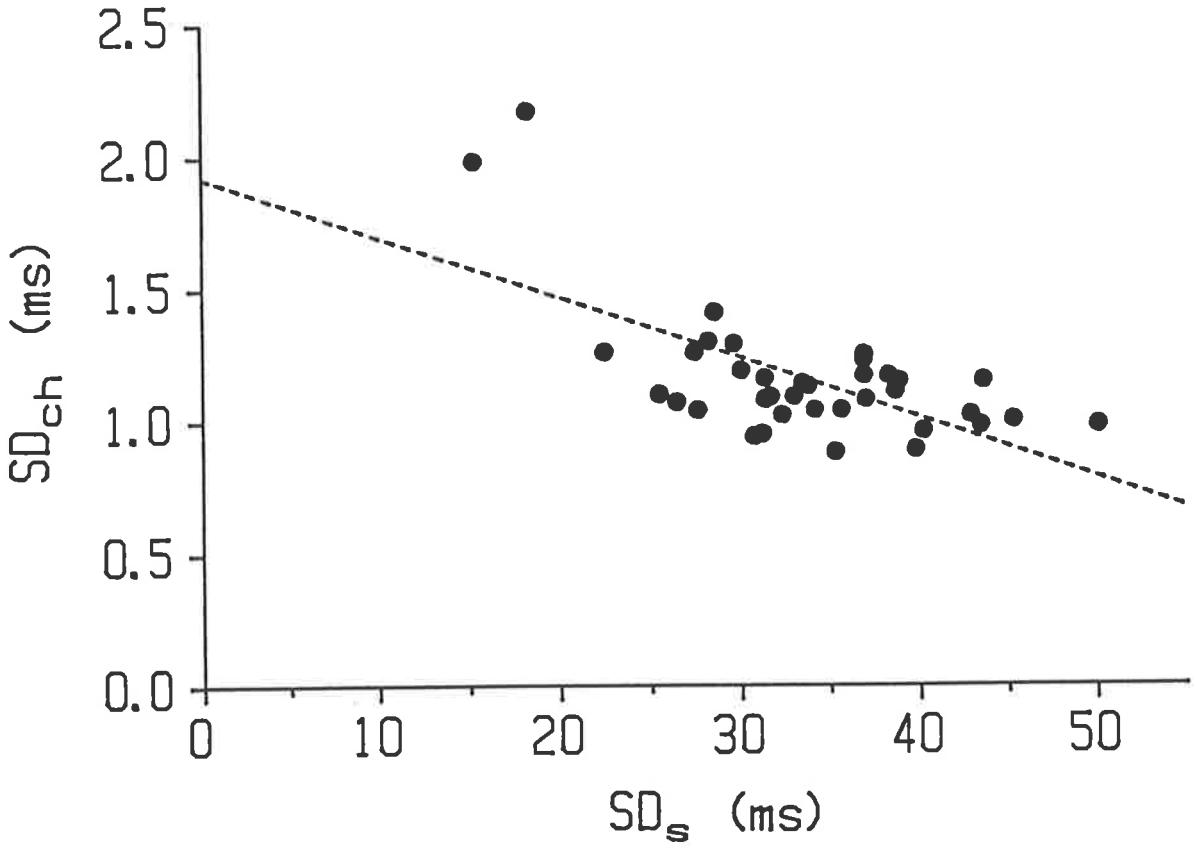

Standard Deviation (ms)
50
40
30
20
10
0
SDe
SDs
0
20
40
60
80
100
120
Mean ISI (ms)

FIGURE 8.2

The correlation between the initial discharge variability and the change in discharge variability over 15 minutes. The initial discharge variability (SD_S) is plotted against the index of the change in the variability with time (SD_{ch}) for each of 37 different motor units. The linear regression line is shown ($r = -0.65$, $P < 0.01$).


2.5
2.0
1.5
1.0
0.5
0.0
SDch (ms)
0
10
20
30
40
50
SDs (ms)

the 15 minutes (SD_{ch}) for the 37 units. In 30 units (81%) the interval variability increased over the 15 minutes (i.e. $SD_{ch} > 1$). There was also a tendency for units with lower initial discharge variability to show the greatest increase in variability over the 15 minutes. Linear regression revealed a significant negative correlation between these variables ($r = -0.65$; $P < 0.001$).

The SD_s values from each of the 37 units were pooled, and compared to the pooled SD_e values for the same units in order to test the statistical significance of the difference in interval variability over the 15 minutes. The mean SD_s for the 37 units was 33.6 $\pm$ 7.2 (SD), and the mean SD_e was 37.7 $\pm$ 6.2 (SD). These values were significantly different (ANOVA, $F = 6.90$; $P < 0.02$), and so it is concluded that for the population of masseter units studied, there was a significant tendency for the variability of discharge at a mean ISI of 100 ms to increase after 15 minutes of continuous activity.

In addition to the 37 units which were controlled by the subject for 15 minutes at a mean rate of 10 Hz, there were an additional 9 units that were controlled for more than 2 minutes, but less than 15 minutes. The SD_s value was also calculated for these units. The 46 SD_s values were grouped by subject and tested in order to detect any subject – dependence of motor unit ISI variability. Five subjects provided SD_s values from 4 or more motor units and were included in the analysis. The data from these units are summarized in Table 8.1. An ANOVA test of these data revealed no significant differences among the subjects for SD_s ($F = 1.81$; $P > 0.05$).

TABLE 8.1

GROUPING OF MOTOR UNIT DISCHARGE VARIABILITY BY SUBJECT.

Subject	mean SD_s	Range of SD_s	no. of units
S.K.	31.3	27.6 – 39.5	12
S.L.	35.7	18.3 – 47.2	9
E.N.	36.8	22.5 – 45.3	8
A.W.	36.9	30.5 – 43.6	5
K.T.	29.4	26.5 – 33.0	4

TABLE 8.2

ANOVA TEST OF THE VARIABILITY OF MOTOR UNIT DISCHARGE FOR DIFFERENT SUBJECTS.

Variable (grouping by subject) vs. variable (SD_S)

Source	*D.F.*	*SS*	*MS*	*F ratio*	*F Prob.*
Between Groups	4	305.7	76.4	1.81	$P > 0.05$
Within Groups	33	1395.3	42.3		
Total	37	1701.0			

Although there were no significant differences in discharge variability when the motor units were grouped by subject, the individual values of SD_S were distributed over a wide range (Table 8.1, also Fig. 8.2), indicating that the variability of discharge with a mean ISI of 100 ms was not uniform for all masseter motor units. In order to investigate the possible physiological significance of this observation, the SD_S was correlated with other measured physiological properties of these motor units.

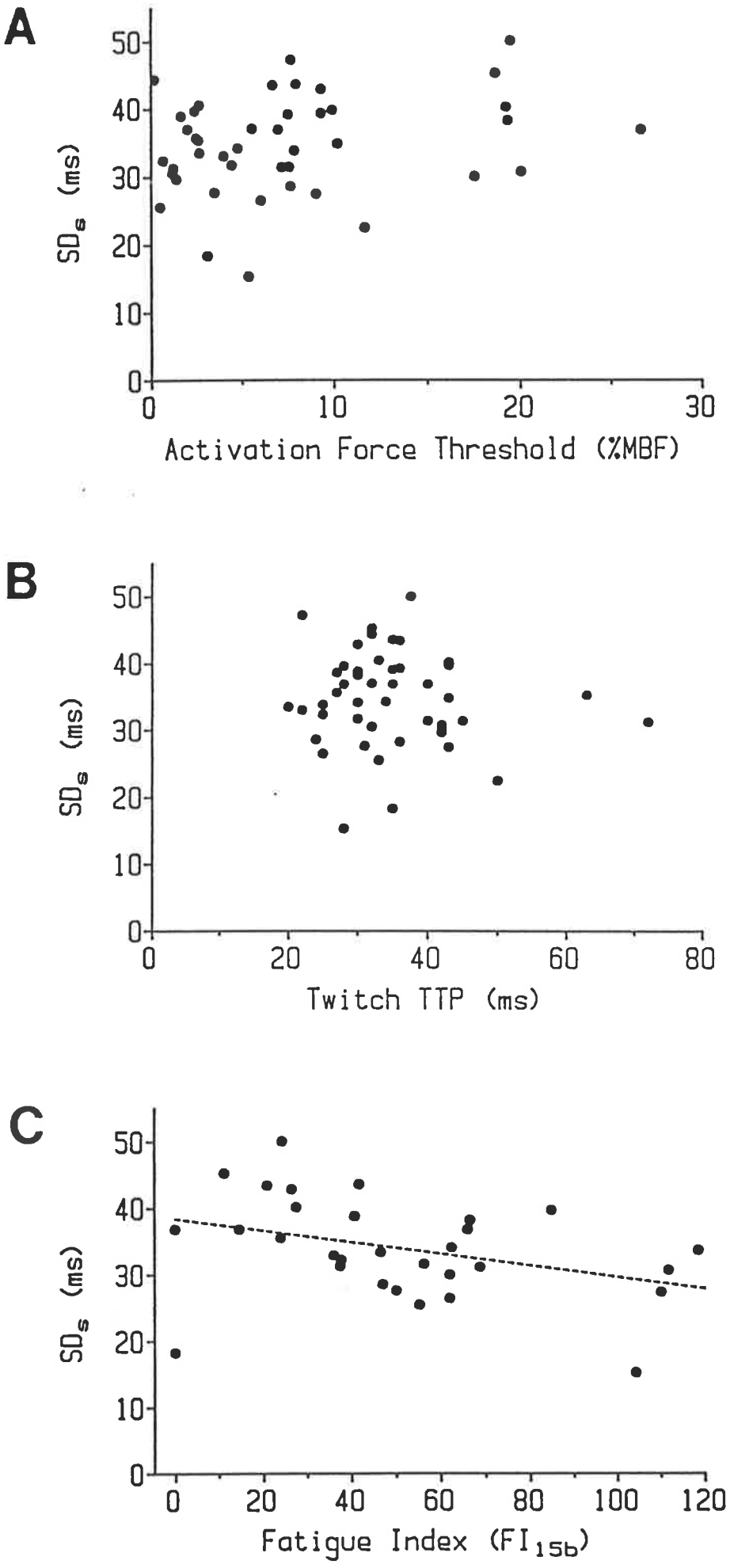
An index of motor unit size is its activation force threshold (Chapter 5). The motor unit's SD_S is plotted against its initial force threshold of activation (as a percentage of the subject's maximal biting force (MBF)) in Fig. 8.3*A*. There was no significant correlation between these two variables ($r = 0.25$; $P > 0.05$, $n = 43$). There was also no significant correlation between the motor unit twitch TTP and SD_S (Fig. 8.3*B*: $r = -0.10$; $P > 0.05$).

The relationship between the motor unit's SD_S and its fatiguability is shown in Fig. 8.3*C*. The fatigue index was the FI_{15b} (see Chapter 5). There was a weak, but significant negative correlation between SD_S and FI_{15b} ($r = -0.37$; $P < 0.05$, $n = 30$). If the aberrant unit with low SD_S and FI_{15b} of 0 is excluded from the regression calculation, the negative correlation is stronger ($r = -0.55$; $P < 0.01$).

In Chapter 6, one possible explanation considered for the change in a motor unit's contribution to the surface EMG during a continuous isometric contraction for 15 minutes was a failure of action potential propagation in some fibres of the motor unit. It was argued that this would result in an increased variability of

FIGURE 8.3

The relationship between the initial discharge variability (SD_S) and 3 physiological characteristics of the motor units. The motor unit's initial discharge variability (SD_S) is plotted against three measured physiological characteristics of the units. These were: *A*, activation force threshold ($n=43$); *B*, twitch TTP ($n=46$); *C*, twitch fatigue index ($n=30$). The linear regression line is shown for *C* ($r=-0.37$; $P<0.05$).


A
50
40
30
20
10
0
SDs (ms)
0
10
20
30
Activation Force Threshold (%MBF)
B
50
40
30
20
10
0
SDs (ms)
0
20
40
60
80
Twitch TTP (ms)
C
50
40
30
20
10
0
SDs (ms)
0
20
40
60
80
100
120
Fatigue Index (FI15b)

motor unit discharge when unit activity was detected by fine – wire intramuscular electrodes, as in the present situation.

Although the discharge variability increased over the 15 minutes for most units, the evidence suggests that the change in firing pattern over time was not related to impairment of electrical propagation in the muscle fibres of the motor unit. In Fig. 8.4*A*, the change in motor unit surface representation amplitude over the 15 minutes is plotted against an index of the change in ISI variability (SD_{ch}) in this period for the 18 motor units from Chapter 6. There was no significant correlation between these variables ($r = 0.25$; $P > 0.05$), indicating that the observed increase in ISI variability over 15 minutes of continuous activity was unrelated to electrical changes in the motor unit. One would expect blocking of fibre action potentials to cause force loss directly, and the poor correlation between SD_{ch} and the unit's Fatigue Index (Fig. 8.4*B*: $r = -0.02$; $P > 0.05$) is further evidence that the increased ISI variability with time was not associated with fibre propagation impairment.

The data obtained from the background units revealed the relationship between the mean ISI and the Standard Deviation of discharge for the masseter units over a wide range of mean ISI's. The pooled data from each of four subjects is shown in Fig. 8.5. Each graph (*A* – *D*) contains data from a number of units firing at different mean rates in the same subject. The data points from each unit were usually confined to a limited range of mean ISI's. Despite the pooling of data, in each case there was a strong tendency for the SD to increase as mean ISI increased, and the data were well fitted by linear regression (the r values for the different subjects ranged from 0.80 – 0.95).

8.4 Discussion.

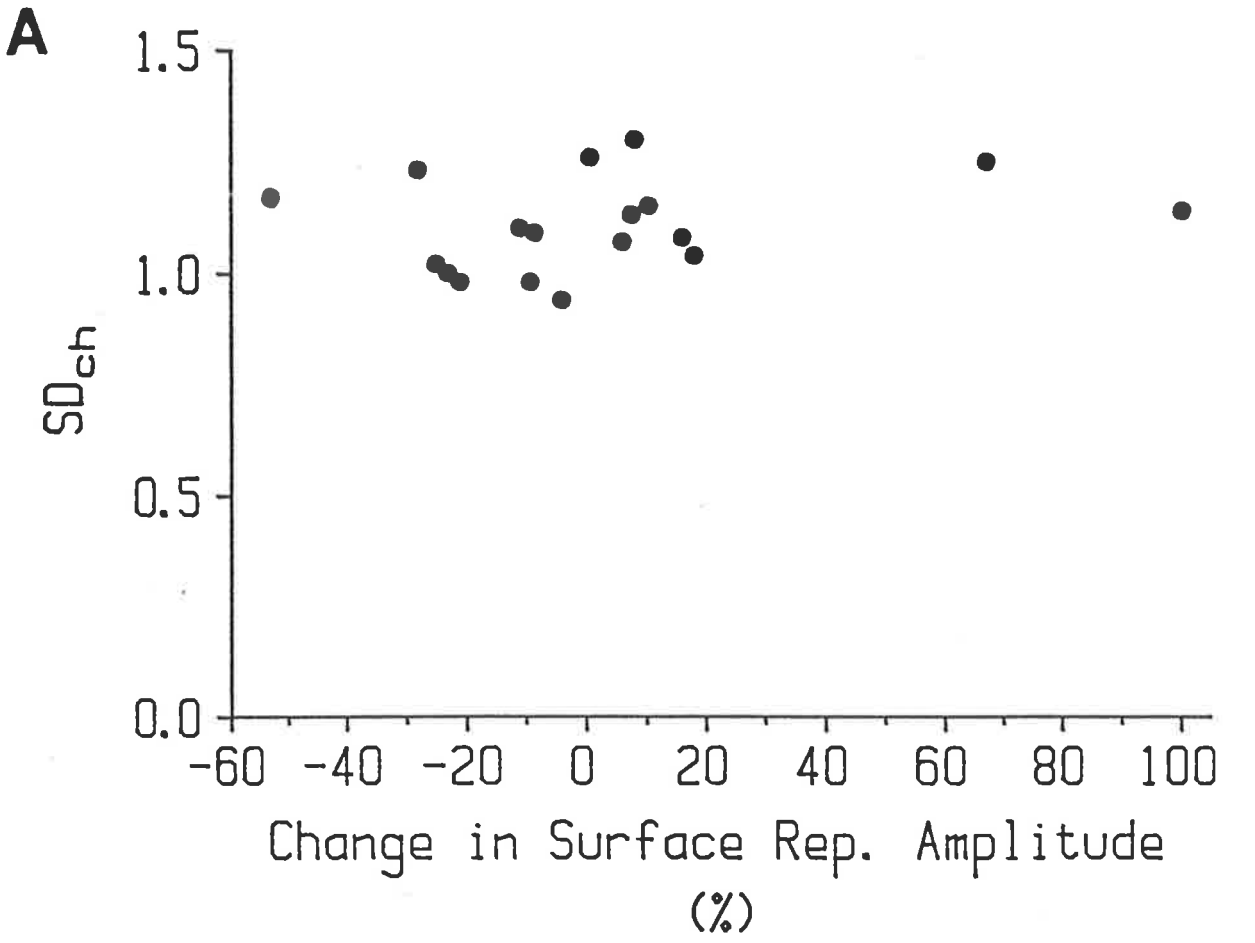
The analyses used in this Chapter resulted in two main findings. The first is that the variability of motor unit discharge in the masseter increased following 15 minutes of continuous activation at a mean rate of 10 Hz. Second, the ISI variability of masseter units in the first two minutes of continuous activity was correlated with their fatiguability.

Factors influencing ISI variability.

Before discussing these results, it is worthwhile to consider the factors determining ISI variability. It has been previously recognized that the regularity of motoneurone discharge at a given rate is influenced by a number of factors, which

FIGURE 8.4

The relationship between the change in discharge variability over 15 minutes and *A*) the change in the motor unit contribution to the surface EMG, and *B*) the unit's fatigue index. *A*: The percentage change in amplitude of the averaged surface representation for the 18 units described in Chapter 6 is plotted against the index of the increase in their discharge variability with time (SD_{ch}). *B*: The fatigue index for each of 30 units is plotted against the SD_{ch}. The fatigue index was FI_{15b} (see Chapter 5).


A
1.5
1.0
0.5
0.0
SDch
-60 -40 -20 0 20 40 60 80 100
Change in Surface Rep. Amplitude
(%)

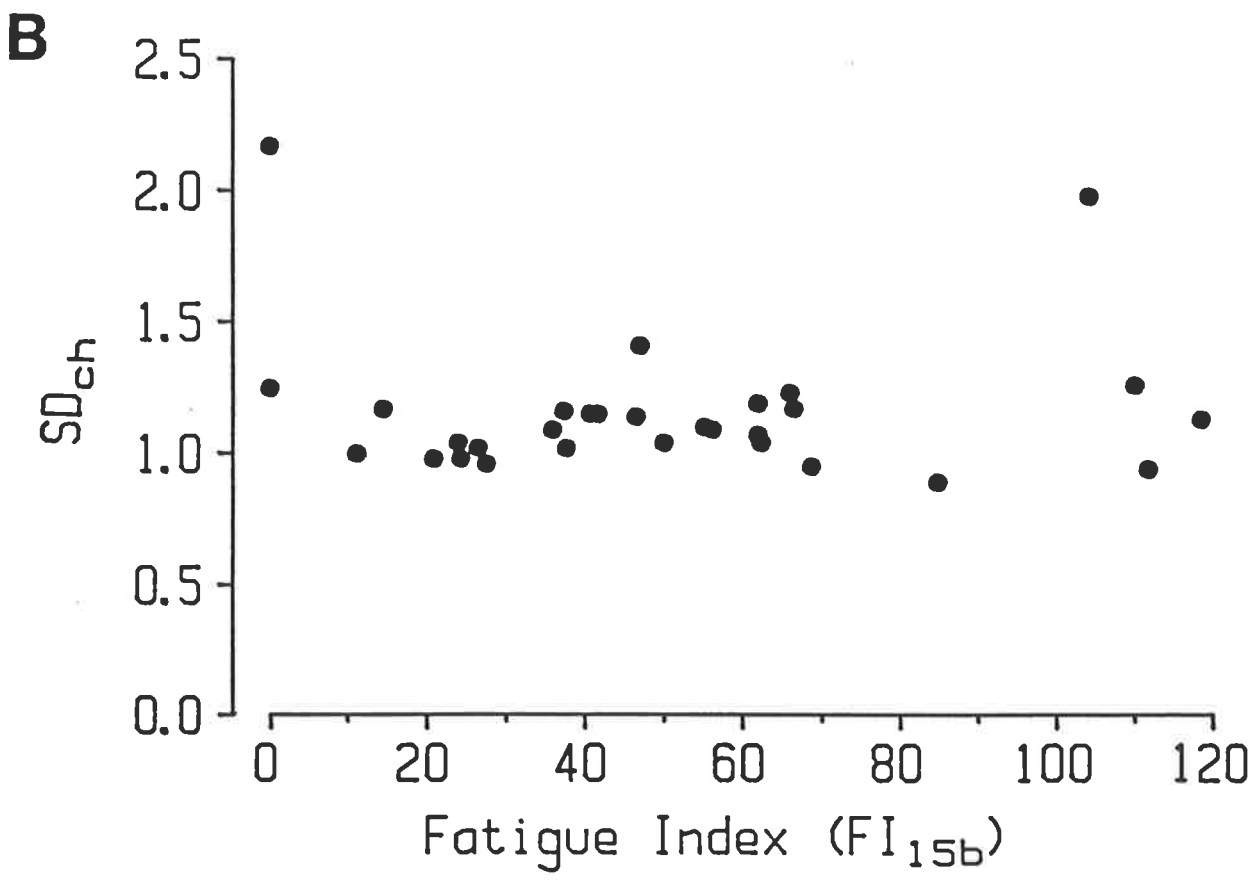
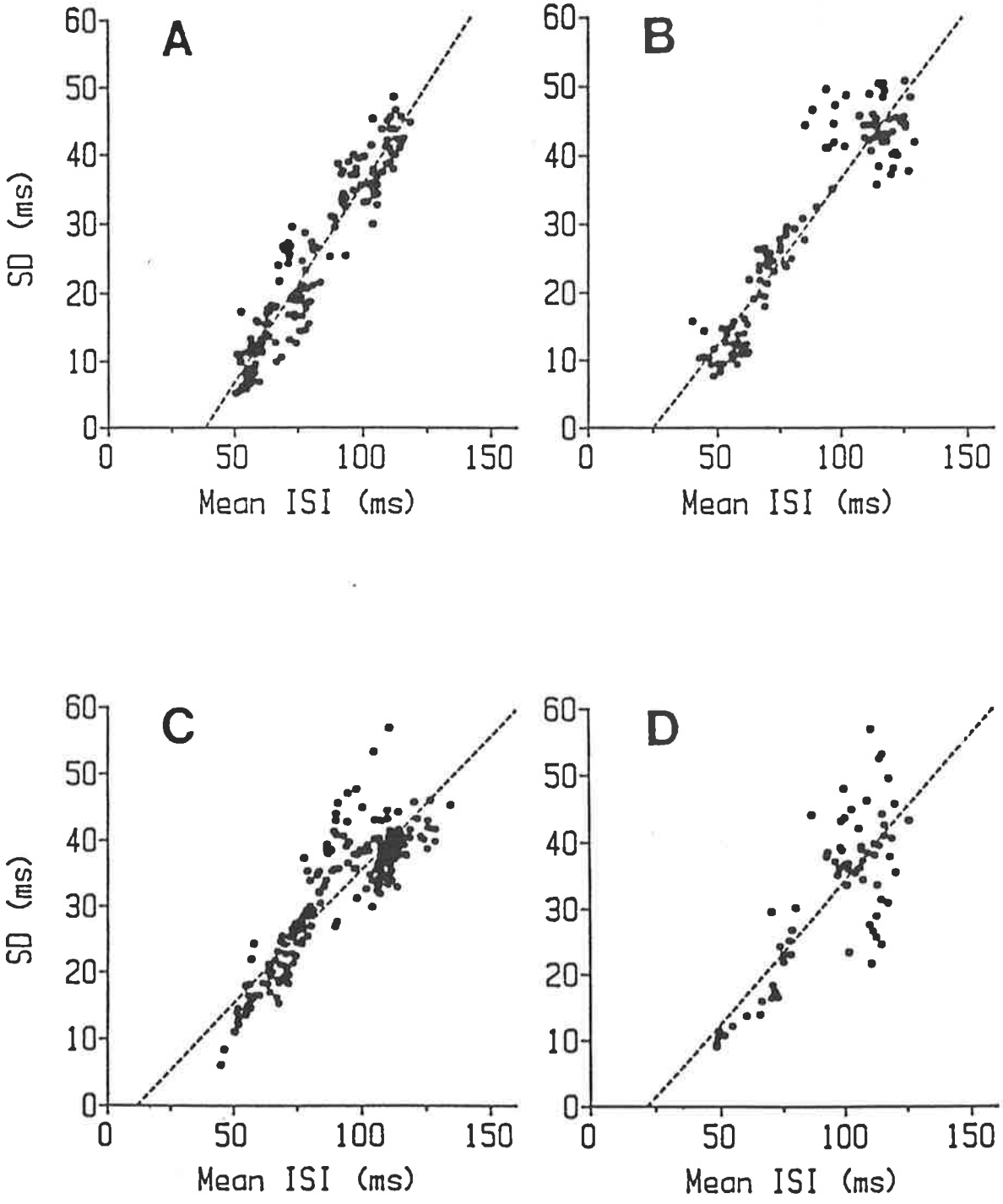

B
2.5
2.0
1.5
1.0
0.5
0.0
SDch
0 20 40 60 80 100 120
Fatigue Index (FI15b)

FIGURE 8.5

The relationship between mean ISI and Standard Deviation of discharge for masseter motor units in 4 subjects. Each graph ($A-D$), contains data from one subject. In each case the mean ISI is plotted against SD for a number of units, and the linear regression line is shown. There are approximately 12 data points from each separate motor unit in each plot. *A* comprises pooled data from 14 units ($r=0.95$); *B*, data from 10 units ($r=0.94$); *C*, data from 17 units ($r=0.89$); and *D*, data from 7 units ($r=0.80$).


A
B
C
D
SD (ms)
Mean ISI (ms)
0
10
20
30
40
50
60
0
50
100
150

include: fluctuations in the threshold firing level of the motoneurone membrane potential, short – term (within an ISI) variability in the synaptic driving current, and synaptic noise in the membrane potential of the motoneurone. Of these, the major influence seems to be synaptic noise as the motoneurone is depolarized, as a result of temporal and spatial summation of a large number of asynchronous post – synaptic potentials (Calvin & Stephens, 1968; Stålberg & Thiele, 1973). Stålberg & Thiele (1973) reported that the addition of a strong reflex input (vibration) to a voluntarily contracting motor unit did not appreciably alter its firing regularity, so short – term variability in the synaptic driving current appears to play little part in ISI variability.

In the study by Stålberg & Thiele (1973), manoeuvres which decreased or increased the afferent inflow to a moderate degree (overstretching the muscle, accessory vibration, cooling) had no measurable effect on the irregularity of motor unit discharge during voluntary contractions. The same result was found by Shiavi & Negin (1975) with muscle stretch. It would seem that the ISI variability is relatively resistant to changes in the pattern of synaptic input, presumably because the enormous number of inputs integrated by the motoneurone means that the properties of the synaptic noise are only affected by gross changes in the number and character of the post – synaptic potentials (e.g. anaesthesia: Stålberg & Thiele, 1973). However, contrary to this view, there is evidence that the regularity of firing of masseter and temporalis motor units may vary with the task performed (Eriksson *et al.*, 1984).

The evidence suggests that synaptic noise can be viewed as the major cause of variation in ISI's. However, there has been little comment in the literature regarding the nature of the relationship between the synaptic noise and the magnitude of the ISI variability. A strong linear correlation between mean ISI and SD has been noted previously for motor units in many human muscles including the masseter. At high firing rates the SD reaches a plateau, so that further increases in rate are not accompanied by a reduction in ISI variability (Tokizane & Shimazu, 1964; Person & Kudina, 1972; Kranz & Baumgartner, 1974; Derfler & Goldberg, 1977). Why is there a linear reduction in ISI variability with increasing mean firing rate (e.g., Fig. 8.5)? Derfler & Goldberg (1977) considered this to be a consequence of a monotonic increase in synaptic input to the motoneurone with a concomitant reduction in the synaptic noise. Several factors suggest that this is unlikely to be the explanation. The first is the relative insensitivity of the ISI variability to changes in synaptic input described above. The second is the observation from the present study that motor units recruited

(and tested) at different levels of voluntary drive do not have systematic differences in ISI variability (Fig. 8.3*A*). If a higher voluntary drive did result in decreased ISI variability, one would expect units recruited at higher forces to have a lower SD_S. This was not the case for the masseter units tested (Fig. 8.3*A*).

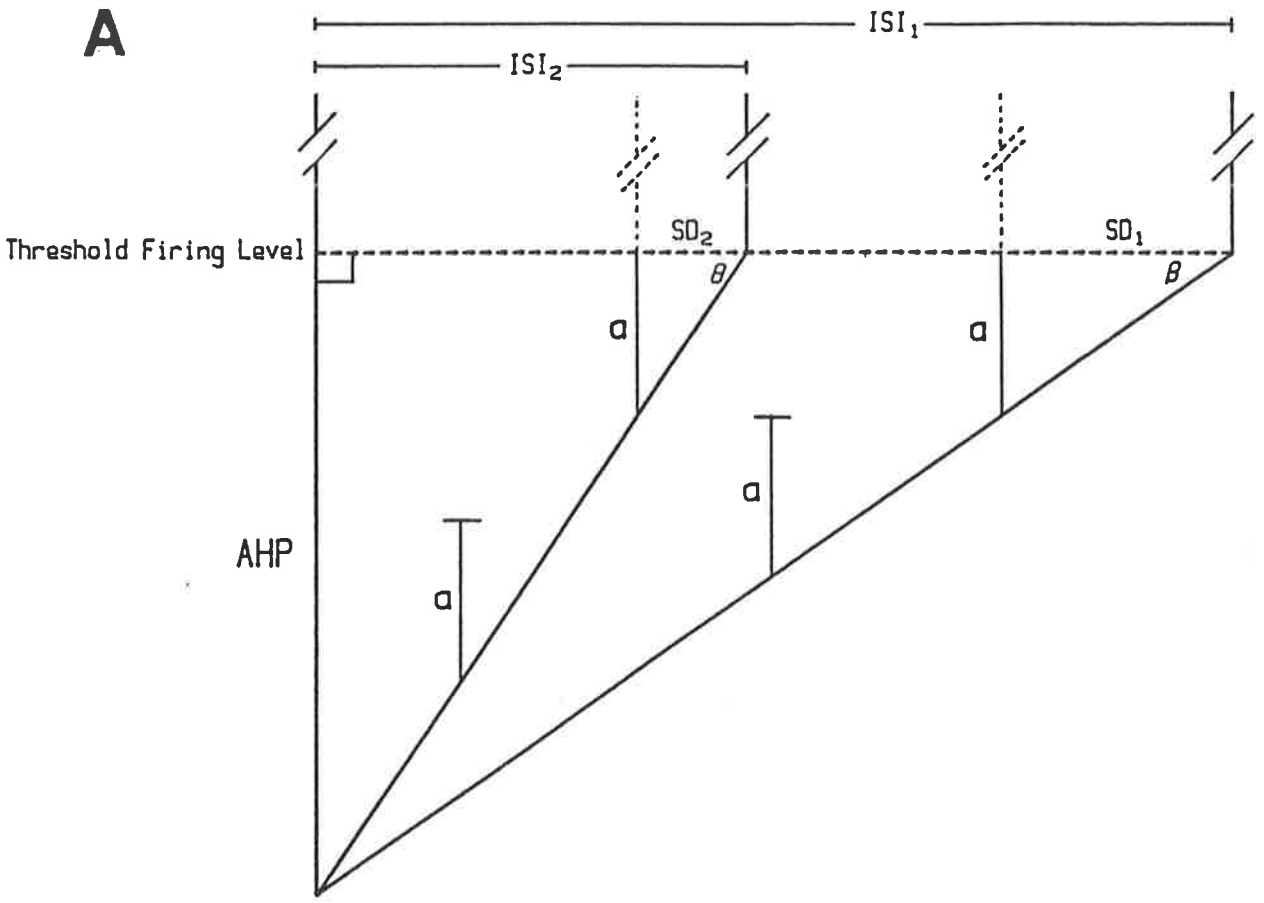
In order to explain the relationship between ISI variability and the mean ISI it is necessary to consider the events occurring in the motoneurone associated with rhythmic firing. The membrane potential trajectories underlying repetitive discharge in human motoneurones are not known with certainty. Schwindt & Calvin (1972) injected current into cat motoneurones and observed the membrane potential trajectories associated with firing at different rates. They found that with currents sufficient to produce repetitive discharge at a rate just above threshold for firing, an increase in rate was accompanied by a increase in the slope of the membrane trajectory. With stronger currents producing higher rates of discharge (but still within the primary range), the slope of the membrane trajectory remained constant, and the rate change was mediated by a change in the "scoop" portion of the AHP.

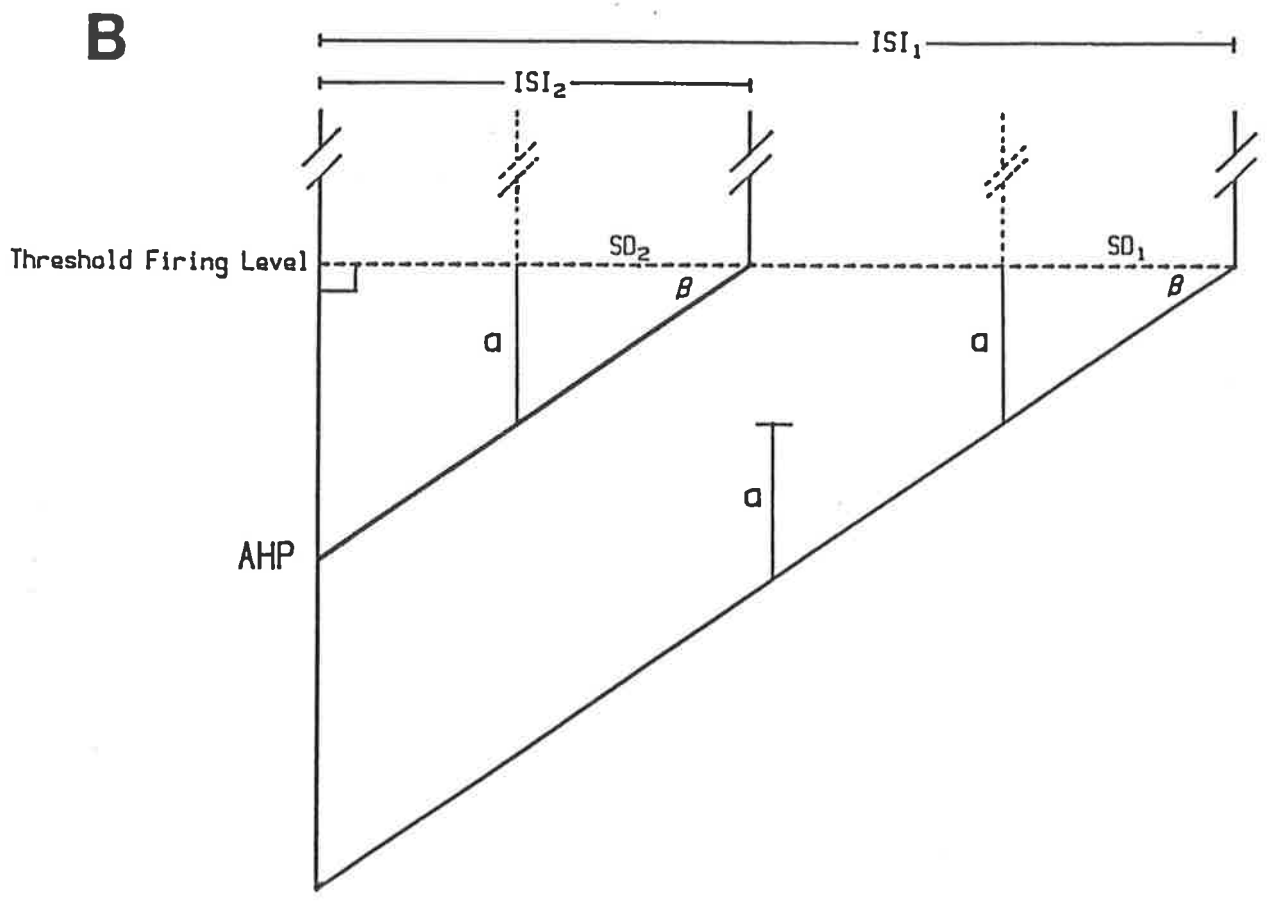
Fig. 8.6 shows a schematic diagram of the effect of the synaptic noise on ISI variability with each of these modes of repetitive firing. With the aid of this model it can be seen that it is not necessary to postulate a change in the properties of the noise to explain the observed relationship between the mean ISI and discharge variability. In *A*, the AHP is constant and a change in ISI is brought about by a change in slope of the depolarization trajectory. The error bars labelled "a" represent the limit of uncertainty in the membrane potential resulting from noise. For clarity, only the noise in the depolarizing direction is shown. In the absence of noise, there would be no variability in successive ISI's with the same membrane trajectory slope. However, the introduction of noise of a finite amplitude causes a variability in the point at which the trajectory reaches firing threshold. The effect of the noise represented here is to advance the phase of the next spike. Noise in the opposite direction (not shown) would tend to retard the phase of the next spike, leading to a distribution of ISI's around the mean ISI. The magnitude of this variability in the ISI is limited by the magnitude (and frequency content) of the noise. However, for a constant level of noise, the magnitude of ISI variability (indicated by its SD, in ms) will be much greater when the slope of the membrane trajectory is shallow, as illustrated by the SD_1 associated with ISI_1, compared with SD_2 associated with ISI_2.

FIGURE 8.6

Schematic diagram to illustrate the effect of noise on motoneurone membrane depolarization trajectories on the variability of ISI's. *A*: Constant – AHP "scoop"/altered – slope model of repetitive action potential generation. In this diagram the AHP is constant at different firing rates, and the membrane trajectories for ISI's of different length are indicated by the lines of different slope approaching the threshold potential (horizontal dashed line). Action potentials are indicated by the solid vertical lines (broken to emphasise the difference in vertical scale) starting at the point where the membrane depolarization reaches the threshold firing level. The error bars labelled "a" represent the limit of uncertainty in the membrane potential resulting from noise. For simplicity, the noise is shown for the depolarizing direction only. The magnitude of the variability in the ISI resulting from the noise in the membrane potential is represented here by the position of the dashed action potentials and the value SD. For a constant level of noise, the magnitude of ISI variability is determined by the slope at which the membrane trajectory approaches the firing threshold.

B: Altered – AHP "scoop"/constant – slope model of repetitive action potential generation. This diagram shows the membrane trajectories underlying rhythmic firing as in *A*, except that in this case ISI's of different length are produced by differences in the amplitude of the AHP, with the slope of the membrane trajectory remaining constant. As the angle β is unchanged for ISI's of different length with this model, then with constant noise the value of SD (in ms) remains constant for ISI's of different length, since $\tan \beta = a/SD$.


A
ISI1
ISI2
Threshold Firing Level
SD2
SD1
θ
β
a
a
a
a
AHP


B
ISI1
ISI2
Threshold Firing Level
SD2
SD1
β
β
a
a
a
AHP

Further predictions can be made about the relationship between mean ISI and SD from the model with the aid of the geometry of right-angled triangles:

$$\tan \beta = \text{opposite/adjacent} = AHP/ISI_1 = a/SD_1.$$

$$\text{ie.} \quad SD_1/ISI_1 = a/AHP. \quad (1)$$

$$\text{also } \tan \theta = AHP/ISI_2 = a/SD_2$$

$$\text{or} \quad SD_2/ISI_2 = a/AHP \quad (2)$$

combining (1) and (2)

$$SD_1/ISI_1 = SD_2/ISI_2 \quad (3)$$

That is, the model predicts that the relationship SD/ISI is a constant. In other words, over the range of ISI's which are produced by membrane trajectories of differing slope, the model predicts a linear increase in SD with an increase in mean ISI, given constant synaptic noise.

With higher injected currents (higher firing rates) in Schwindt & Calvin's experiments, changes in rate were accomplished by an altered AHP scoop, with an unchanged slope in the membrane trajectory. The effect of this mode of action potential generation on ISI variability is illustrated in Fig. 8.6*B*. With constant noise under these circumstances, the SD would remain unchanged for ISI's of different duration (i.e., $SD_2 = SD_1$).

In humans, the relationship between mean ISI and SD has been measured in many muscles, and SD is found to decrease linearly with decreasing ISI over a range of mean firing rates above the tonic firing threshold. As the firing rate is increased, a point is reached where a plateau occurs in the SD values, so that an increase in rate beyond this value is not accompanied by a further increase in interval variability (Tokizane & Shimazu, 1964; Person & Kudina, 1972; Derfler & Goldberg, 1977). In the masseter, the plateau begins at high rates (above 20 Hz; Derfler & Goldberg, 1977), and was therefore not evident in the data shown in Fig. 8.5. On the basis of the model in Fig. 8.6, these observations of the ISI/SD relationship provide strong circumstantial evidence that the firing-rate control of human motoneurones is mediated in precisely the same manner as the cat motoneurones responding to intracellularly injected currents in Schwindt & Calvin's experiments. At low firing rates (longer ISI's), the slope of the membrane trajectory is shallow, and hence a given level of synaptic noise produces a large ISI variability. At higher rates the slope becomes steeper with an unchanged

AHP, and ISI variability decreases linearly (Fig. 8.6*A*). At still higher rates the action potential generating mechanism switches to the altered – scoop/constant – slope type, at which point ISI variability reaches a plateau, because the depolarization slope is unchanged for further increases in rate.

Direct evidence for a change in the depolarization ramp slope with changing firing rate in human soleus motor units has recently been found in another study in this laboratory (personal communication from Miles, Türker & Le). In these experiments, the same proportion of spikes (normalised with respect to the ISI) were phase – advanced by a constant H – reflex stimulus in a motor unit tested at different mean rates. Their data were consistent with a model in which the amplitude of the AHP remains constant at different firing rates, while the slope of the linear membrane potential trajectory varies. The H – reflex method of Miles *et al.* offers a way of testing the hypothesis that the ramp – slope mechanism is replaced by the altered – scoop/constant – slope mechanism of firing rate modulation at high rates in humans. If the hypothesis is correct, testing the reflex in a motor unit firing at rates higher than that where the plateau in the ISI/SD relationship starts should reveal a quantitative difference in the normalized H – reflex response at different rates, compared with the constant responses obtained in the same unit when tested at lower rates. Testing in the same manner in different muscles should help to explain the variation between muscles of the point at which the plateau in the ISI/SD relationship begins (Tokizane & Shimazu, 1964).

In summary, it appears that the major determinants of ISI variability at a given mean ISI are the synaptic noise and the steepness of the slope of the depolarization trajectory as the membrane potential nears the firing threshold. Of the two, the synaptic noise induces variability in ISI's, and appears to be relatively invariant under normal conditions. The magnitude of the effect of the synaptic noise on ISI variability seems to be determined by the slope of the depolarization curve.

The increase in ISI variability with prolonged activation.

It is not possible at the moment to identify the cause of the increased variability with time in the present study, yet there are several lines which are worth pursuing. There were no consistent changes in either the total force or in the firing patterns of other active units during these contractions (see Chapter 9) which might suggest a consistent change in afferent input to the motor units under investigation. In view of the relative insensitivity of ISI variability to

changes in afferent input (discussed earlier), it therefore seems unlikely that the observed increase in ISI variability arose from a change in the synaptic noise. The negative correlation between the initial discharge variability of the motor unit and the change in variability with time (Fig. 8.2) points to a unit – specific cause, and in view of the high degree of convergence of synaptic inputs to motoneurones, it is difficult to explain this phenomenon in terms of synaptic noise.

The more likely hypothesis therefore seems to be that a change in intrinsic motoneurone properties occurs during prolonged activation which makes the slope of the depolarization trajectory shallower at a mean ISI of 100 ms, with an accompanying increase in ISI variability. Current injection experiments such as those of Schwindt & Calvin (1972) demonstrate that spike generating mechanisms exist within the motoneurone (*viz.* a change in the AHP scoop) which could allow such a scenario. There is no information about the effects of repetitive firing for 15 minutes on the properties of motoneurones in experimental animals, because technical limitations restrict most current injection experiments to less than a few minutes of repetitive firing.

This hypothesis is testable in humans using the H – reflex method of Miles *et al.* If the depolarization trajectory does change for a given ISI in motor units with prolonged activation, differences should be revealed in the unit's response to H – reflex testing at the same mean rate over the duration of the contraction. Specifically, the normalized H – reflex should be greater at the end of the contraction than it was at the beginning. The differences in the H – reflex should also correlate with the change in ISI variability for that unit during the period of voluntary activation.

The high discharge variability of masseter motor units

In agreement with Derfler & Goldberg (1977), the discharge variability of masseter motor units at a given mean ISI was found to be high (Fig. 8.5), when compared to the limb muscles. Similarly high discharge variability was also noted for other facial muscles innervated by cranial motoneurones by Tokizane & Shimazu (1964). The authors of these studies considered that the less – regular discharge in these muscles reflected differences in synaptic input, such as the lack of recurrent inhibition in the trigeminal system. However, because of the enormous number of synapses to motoneurones from other sources, it does not seem likely that the absence of these inputs markedly affects the synaptic noise.

A more likely possibility, based on the model presented in the previous section, is that the higher ISI variability reflects differences in the biophysical properties of masseter motoneurones, such as the AHP, which result in a shallower membrane depolarization trajectory for a given mean ISI in the masseter units.

Correlation between motor unit discharge patterns and physiological properties.

Previous studies in humans have found correlations between motor unit discharge patterns and muscle histochemical fibre type (Warmolts & Engel, 1972), recruitment threshold (Hannerz, 1974), and axonal conduction velocity (Borg *et al.*, 1978). It is known that motoneurones belonging to motor units of different contractile speed have differences in their AHP (Eccles *et al.*, 1958), and it has been suggested that AHP has a role in the control of firing regularity (Person & Kudina, 1972). From these results one would expect some differences in discharge patterns of motor units with different physiological properties.

In the present study, no significant correlations were found between ISI variability and either motor unit recruitment threshold or twitch TTP for the masseter units (Fig. 8.3*A*,*B*). It is concluded that for the population of masseter motor units studied (predominantly recruited at less than 20% MBF), neither the size nor contractile speed of the motor unit was related to the variability of discharge at a mean ISI of 100 ms. With regard to recruitment threshold, the poor correlation may be partly due to the fact that the range of recruitment thresholds of tested units was lower in the masseter than in the study by Hannerz (1974) in anterior tibialis. Alternatively, it may reflect differences between muscles, as a poor correlation between firing regularity and recruitment threshold was also reported by Freund *et al.* (1975) in human FDI over a wider range of relative recruitment forces than the present study. The unusual properties of the human masseter have already been described in Chapter 5, and features such as the paucity of type S motor units, a relatively uniform motor unit population in terms of twitch TTP, and the poor correlation between motor unit size and twitch TTP, may serve to weaken expected correlations between motoneurone discharge patterns and motor unit physiological properties in the masseter.

There was, however, a correlation between the regularity of a masseter motor unit's discharge in the first two minutes of activity, and its fatiguability (Fig. 8.3*C*). The first consideration is whether the observed correlation between initial ISI variability and fatigue may be an artifact of the STA technique. As interval variability increases, there are more long and short intervals for a given mean ISI. This is a potential source of error when the STA technique is used to measure

twitch fatiguability, as the twitch is influenced by the firing pattern of the unit (Chapter 4). With the spike parameters used for valid triggers (300 – 100:100 – 300), an increase in the proportion of short intervals is not a problem as pre – and post – trigger intervals below 100 ms were not allowed for valid trigger spikes. However, an increased variability would result in a greater proportion of long pre – and post – trigger intervals for the valid spikes used for the average. This could potentially give a less – fused twitch under these conditions, which might lead to an underestimation of twitch fatigue.

However, if the change in ISI variability with time did have an important influence on STA twitch amplitude, one would expect a strong correlation between SD_{ch} and the fatigue index. This was not the case, and in fact there was no significant correlation between them (Fig. 8.4*B*). The poor correlation between the change in motor unit force output and the change in discharge variability over the 15 minutes suggests that ISI variability *per se* and the STA twitch force were not directly related.

Therefore, it is concluded that motor units with less – regular firing patterns at a mean ISI of 100 ms are more susceptible to fatigue in the human masseter. Indeed, in the present series of experiments, ISI variability was a better predictor of masseter motor unit fatiguability than properties such as motor unit size and contractile speed (Chapter 5). This can be viewed as a link between the discharge pattern of the motoneurone and a physiological property of the muscle fibres it controls. Evidence for such a link has been found in other muscles. It has been shown that the firing pattern of motor units can be altered by training which alters the mechanical properties of the muscle (and, by inference, its motor units). In a long – term study of the anterior tibialis, Cracraft & Petajan (1977) found that motor units fired less regularly following a program of high – intensity, short – duration exercise (strength training), whereas motor unit discharge was more regular following low – intensity, long – duration exercise (endurance training). It remains to be seen whether the link between motoneurone discharge variability and muscle represents a property of the motoneurone itself which is inter – related with the functional properties of the muscle fibres it innervates, or whether other factors (e.g. pre – synaptic) are involved.

CHAPTER 9

MOTOR UNIT FIRING PATTERNS DURING PROLONGED ISOMETRIC CONTRACTIONS.

9.1 Introduction.

The control of muscle force is achieved during voluntary contractions by variation of the number of motor units active (recruitment), and by modulation of the firing pattern of the active motor units. From the many studies of the recruitment properties of motor units in both animals and humans, an understanding has emerged of the strategies used by the CNS to activate the motor units to produce force. During force – varying contractions motor units are recruited and de – recruited in an orderly sequence which correlates with their size. The hierarchy of recruitment is relatively fixed for repeated contractions under most circumstances. This has become known as the "size principle" of recruitment (reviewed by Henneman & Mendell 1981).

The firing patterns of individual motor units have not received the same attention as their recruitment properties. Most studies have been limited to contractions of short duration and have examined the firing rates of active units during force – varying contractions. It has been observed that gross variation in the force output of a muscle is accompanied by a roughly proportional change in the firing rate of all the active motor units in the muscle (Monster & Chan, 1977; Person & Kudina, 1972; Milner – Brown *et al.*, 1973*c*), and that at least in some muscles the firing rate at recruitment for all units is similar (Milner – Brown *et al.*, 1973*c*). The corollary of this is that the recruitment hierarchy is reflected in the firing rate hierarchy of the active units in a short – term contraction; the last – recruited units are not only larger, but also have a lower discharge rate than the earlier – recruited units (Tanji & Kato, 1973*b*; Derfler & Goldberg, 1977; Miles & Türker, 1987).

There is evidence that the normal motor unit recruitment order can be altered under certain conditions, for example during a change in cutaneous afferent input to the motoneurones (Kanda *et al.*, 1977; Kernell & Sjöholm, 1975; Garnett & Stephens, 1981). However, there is little information regarding analogous changes in motor unit firing patterns during prolonged activation. This undoubtedly

reflects the technical difficulty of following the activity of the same motor unit(s) for long periods.

Most previous studies of motor unit firing patterns during continuous activation have followed the mean firing rate of an isolated single unit during a constant – force paradigm (Smith 1934, Lindsley 1935, Bigland & Lippold 1954, Freund *et al.* 1975; Person & Kudina 1972, Kranz & Baumgartner 1974, Bigland – Ritchie *et al.*, 1985). These studies give little information regarding a change in functional properties of the motor units with time, because of the unreliability of using the total force as an index of the excitatory drive to the units. A change in motor unit firing rate while the force is kept constant does not necessarily indicate an altered response to a constant excitatory drive, because the force output of a muscle may change independent of the central drive or effort, particularly in contractions of long duration (e.g., due to contractile fatigue). In addition, the force measured is invariably the net torque around a joint. Co – contraction of antagonist and synergist muscles affects the net torque applied, and the relative contribution of other muscles may change during the contraction so that the net torque is not proportional to the net excitatory drive to the motor units under study.

The issue that has not been addressed in the previous studies is whether the hierarchy of motor unit firing rates found in short – term isometric contractions, and reflecting the recruitment order, is preserved during contractions of long duration. In the present study the firing rate of one motor unit in the muscle was controlled voluntarily by the subject at a constant prescribed rate, and the firing pattern of 1 – 4 concurrently – active units was monitored during a continuous isometric contraction of long duration (usually 15 minutes). With this method the net excitatory drive to one of the active motor units was controlled, and this was used as the reference for the functional state of the other active units.

9.2 Methods.

Apparatus and recording procedure.

These arrangements were identical to those previously described in Chapters 2 & 3.

Protocol.

This was identical to that described in Chapter 5. The subject controlled the mean firing rate of a selected unit at 10 Hz for 15 minutes during a continuous isometric contraction with the aid of audio and visual feedback. The unit controlled by the subject will be referred to hereinafter as the "feedback" unit. In most cases the feedback unit was one of the units whose fatigue data were presented in Chapter 5. While the subject controlled the feedback unit at 10 Hz it was usually possible to detect the activity of other units in the same, or separate intramuscular electrodes: these units are referred to as "background" units. Subjects received no feedback regarding the activity of background units.

Analysis.

The intramuscular EMG records were analysed off-line to determine the firing pattern of all the single units recorded during the contraction. Motor unit action potentials in each record were discriminated using the SPS 8701. Only individual spike trains that could be discriminated with high reliability were used for analysis. Intramuscular records containing action potentials from only one or two motor units were invariably satisfactory. In records containing action potentials from three or more motor units, recognition errors due to action potential superimpositions can become significant (see Chapter 2). An accurate estimate of the total number of missed spike recognitions was readily available when the SPS 8701 was used to identify motor unit action potentials. Following each run, the number of unidentified waveforms with an amplitude sufficient to trigger the discriminator was displayed on the screen. Unidentified waveforms were stored, and could be viewed following a run (a very useful facility to check that the stored template had the appropriate tolerance in order to minimise false negative recognition errors). Following trial runs to optimise the recognition parameters, the data were rejected if the intramuscular record could not be discriminated with less than 5% of total spikes unclassified due to recognition errors (almost exclusively superimpositions). This usually restricted analysis to the 3 units with the largest amplitude in any particular record, and a maximal permissable error of approximately 3% in missed recognition of spikes from any one unit (of three).

In most cases, the motor unit action potential waveform changed over the 15 minutes. Units were only included in the analysis if any change in action potential waveform was observed to occur slowly and progressively. Units with abrupt changes in waveform were excluded. Identification of unit potentials was confirmed independently by three investigators.

The interspike intervals (measured to the nearest millisecond) for each discriminated unit were stored, and the SPS 8701 was used to calculate the mean interspike interval (ISI) and standard deviation (SD) for each single unit over successive 30 – second epochs during the 15 – minute contraction. Stationarity of the pulse trains during each 30 – second epoch was checked by visual inspection of the instantaneous firing rate records. Only data without definite trends in rate during an epoch were used for statistical analysis.

An interval exceeding the mean ISI by more than 3 standard deviations in any spike train was assumed to have arisen from missed recognition of an action potential by the SPS 8701, and was excluded from the final calculation of the mean ISI and SD for that epoch. This minimised the effects of recognition errors on the firing rate statistics.

The procedures used to test the statistical significance of any changes in mean ISI with time were as follows:

i) The feedback unit was examined first to ensure that its mean ISI from a 30 – second epoch in the first minute of the contraction did not differ significantly from the required value of 100 ms, using a t – test. A P value of less than 0.01 was deemed to indicate a significant difference in mean ISI for the results presented in this Chapter.

ii) The feedback unit was then tested to ensure that the mean ISI obtained in the first minute of the contraction and also from a 30 – second epoch in the 15th minute of the contraction were not significantly different ($P > 0.01$) by a t – test.

iii) Following verification that the mean ISI of the feedback unit was acceptably close to 100 ms, and not significantly altered with time, the mean ISI $\pm$ SD was calculated for each background unit from the identical 30 – second epochs at the start and end of the contraction as above. For each background unit, the mean ISI's from the first and 15th minute of the contraction were tested for a significant difference with a t – test.

In this Chapter the firing pattern of the motor units is frequently described in terms of mean firing rate rather than mean ISI. This is primarily because this means of describing unit activity is a more familiar one. However, all statistical procedures were performed using the mean ISI and SD.

9.3 Results.

Provided that the subjects received reliable feedback, they were able to control the firing rate of the selected unit without difficulty for the 15 minutes of the contraction. Off-line analysis showed the mean firing rate of the feedback unit was usually less than 10 Hz, but rarely differed significantly from this value in any 30-second epoch. Each background unit that could be discriminated with high accuracy was paired with its corresponding feedback unit for comparison of their firing patterns throughout the contraction.

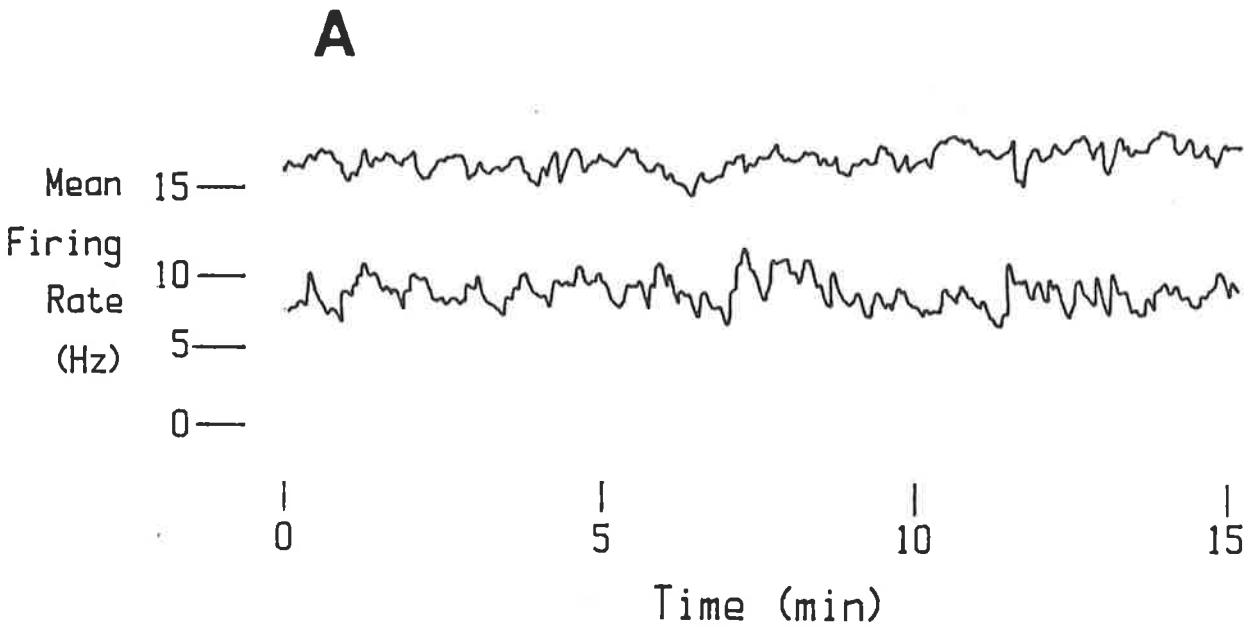
The behaviour of the background units over the long term was not uniform. In Fig. 9.1*A*, for example, the feedback unit was maintained at a constant rate by the subject, and the background unit continued to discharge at a mean rate of 16 Hz for the 15 minutes. Segments of the intramuscular EMG record from which these two units were discriminated are shown in Fig. 9.1*B*. The larger-amplitude unit is the one which was controlled at 10 Hz by the subject. These two units were the only units whose activity was detected on this electrode, and were unambiguously discriminated throughout the 15 minutes of the contraction.

In contrast to this pattern, in other pairs of units, the firing rate of the background unit did not remain constant with time. An example is shown in Fig. 9.2*A*. In this case, the firing rate of the background unit (Unit *b*) was actually less than that of the feedback unit (*a*) in the initial part of the contraction, yet the firing rate of Unit *b* increased gradually to peak at about 13 Hz after 8 minutes of continuous activity, and this rate was maintained for the remainder of the contraction. The intramuscular EMG record is shown in Fig. 9.2*B*. The larger amplitude unit was the feedback unit. The action potential of Unit *b* was of intermediate amplitude in the 1st minute, and there was a third unit with a smaller action potential also visible. By the 15th minute the action potentials of each unit had decreased in amplitude, yet units *a* and *b* were still clearly discriminable. The ISI's of unit *b* were shorter in the 15th minute.

Thirty-three feedback/background unit pairs were followed for the full 15 minutes. In each case, the mean ISI of the feedback unit did not change significantly with time. The firing patterns of the background units were tested to determine the statistical significance of any change in mean ISI between the first and 15th minutes. In 16 background units (48%) the mean ISI was not significantly different in the first and 15th minute; in 11 background units (33%) the mean ISI decreased (firing rate increased) significantly; and in 6 pairs (19%) the mean ISI increased (firing rate decreased) significantly.

FIGURE 9.1

Example of a pair of masseter units which maintained their relative firing rates for 15 minutes. The mean firing rate is plotted against time for two units in *A*. The firing rate records have been heavily low-pass filtered in order to show long-term trends more clearly. The lower trace belongs to the feedback unit. Both the background unit and the feedback unit maintained their mean firing rates at a constant level for 15 minutes. Segments of the intramuscular EMG records from the first and 15th minute of the contraction are shown in *B*. The feedback unit has the action potential with the largest amplitude. The action potentials from both units were readily discriminated throughout the contraction.


A
Mean
Firing
Rate
(Hz)
15
10
5
0
0
5
10
15
Time (min)

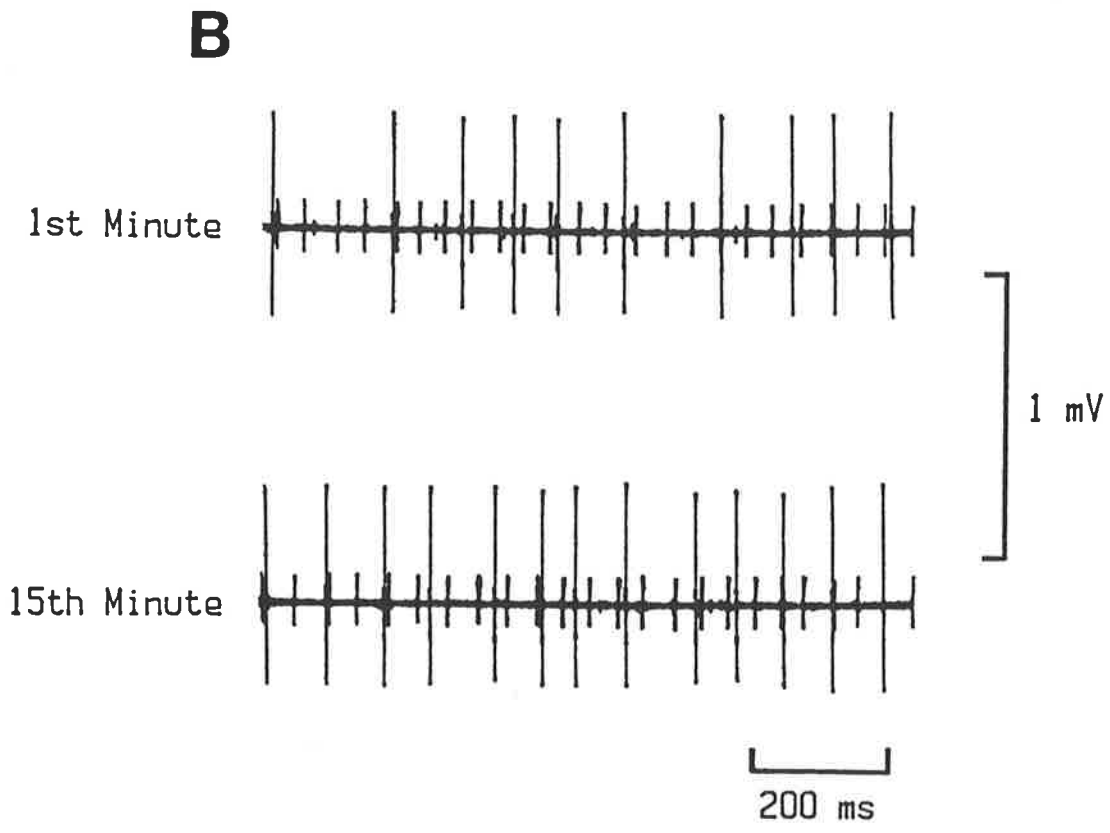
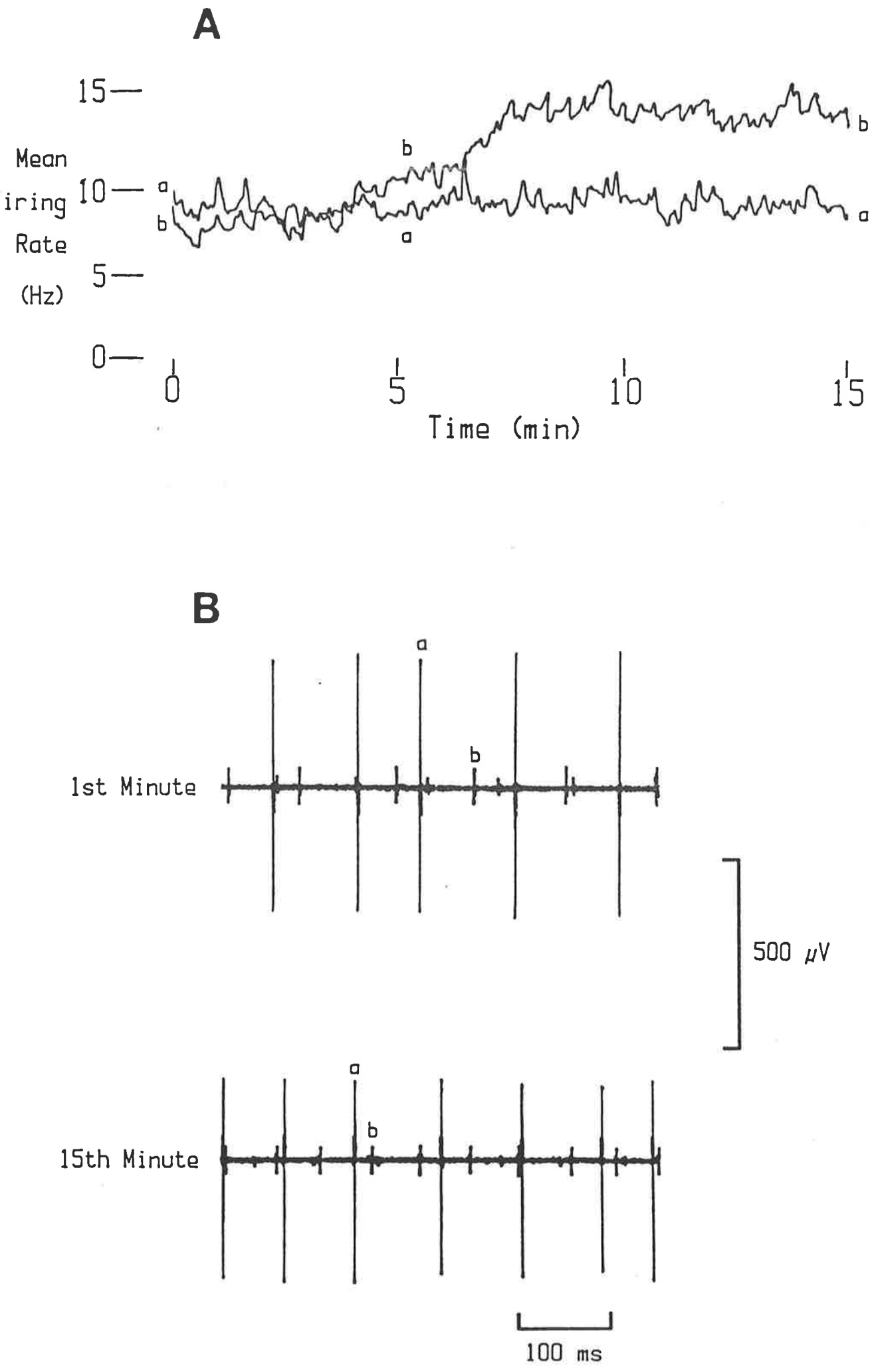

B
1st Minute
15th Minute
1 mV
200 ms

FIGURE 9.2

Example of a pair of masseter units demonstrating differential firing rate behaviour, and a reversal of the original rank order during 15 minutes of activity. In *A*, the mean firing rate is plotted against time for two units. The firing rate records have been heavily low – pass filtered to illustrate slow trends in mean rate more clearly. The trace belonging to the feedback unit is marked *a*, and its mean rate remained unchanged for the 15 minutes at just below 10 Hz. The background unit (trace *b*) initially fired at a mean rate below that of the feedback unit, but the rate increased slowly until it reached 13 Hz after 7 minutes, and then remained steady. Segments of the intramuscular EMG records from the first and 15th minute of the contraction are shown in *B*. The feedback unit has the action potential with the largest amplitude. The action potentials from both units were readily discriminated throughout the contraction, although there was a change in amplitude with time.


A
15
Mean
iring
Rate
10
(Hz)
5
0
a
b
b
a
b
a
0
5
10
15
Time (min)
B
1st Minute
a
b
500 μV
15th Minute
a
b
100 ms

On a number of occasions additional background units could not be reliably discriminated for the full 15 minutes, due to changes in action potential waveform or abrupt cessation of activity, presumably resulting from electrode movement. These units were excluded from the analysis. However, there were 10 background units that ceased firing before the end of the test period, but could be re-activated later by a more forceful bite. In 8 of these units there was a progressive reduction in firing rate prior to the cessation of firing, and the mean firing rate in the last full 30-second epoch in which the unit was tonically active was significantly below its initial mean rate. In the other 2 units, the initial firing rate was too low for a significant change in rate to be evident before the unit ceased firing. The evidence suggests that in these 10 units the drop-out was not due to electrode movement, but rather that the unit ceased firing because the net excitation fell below the unit's functional threshold.

These 10 units were therefore included in the analysis, and the distribution of the changes in mean firing rate over the 15 minutes becomes: In 18 background units (42%) the mean ISI did not change significantly; in 11 background units (26%) the mean ISI decreased significantly (firing rate increased); and in 14 pairs (32%) the mean ISI increased significantly (firing rate decreased). The changes in firing rate for the 43 background units are summarized in the histogram in Fig. 9.3. The threshold for a significant change in rate was about ± 1 Hz, regardless of the initial firing rate. The largest change of rate observed in a background unit was an increase of 6.6 Hz.

It was often possible to detect more than one background unit during the same contraction. The mean firing rate behaviour of a background unit was relatively independent of the behaviour of other background units. This can be seen by reference to Table 9.1. The even distribution of units falling in each category in Table 9.1 does not suggest any link between the firing rate behaviour of both background units over the 15 minutes, although the number of pairs in each category was insufficient for a statistical test. Nevertheless, in only 3 cases in 24 (12.5%) did both background units of a pair behave in the same manner as the feedback unit; i.e., no significant change in mean firing rate after 15 minutes. This is consistent with the proportion expected (11%) if it is hypothesized that the change in mean firing rate of the three motor units were independent variables.

FIGURE 9.3

Histogram showing the distribution of the change in firing rate with time amongst the background units. Each count represents the change in mean firing rate of a background unit over 15 minutes while the feedback unit was maintained at 10 Hz. Differences in mean firing rate that were not significant are indicated by the open bars, and changes in rate that were significant ($P < 0.01$) are given by the shaded bars.

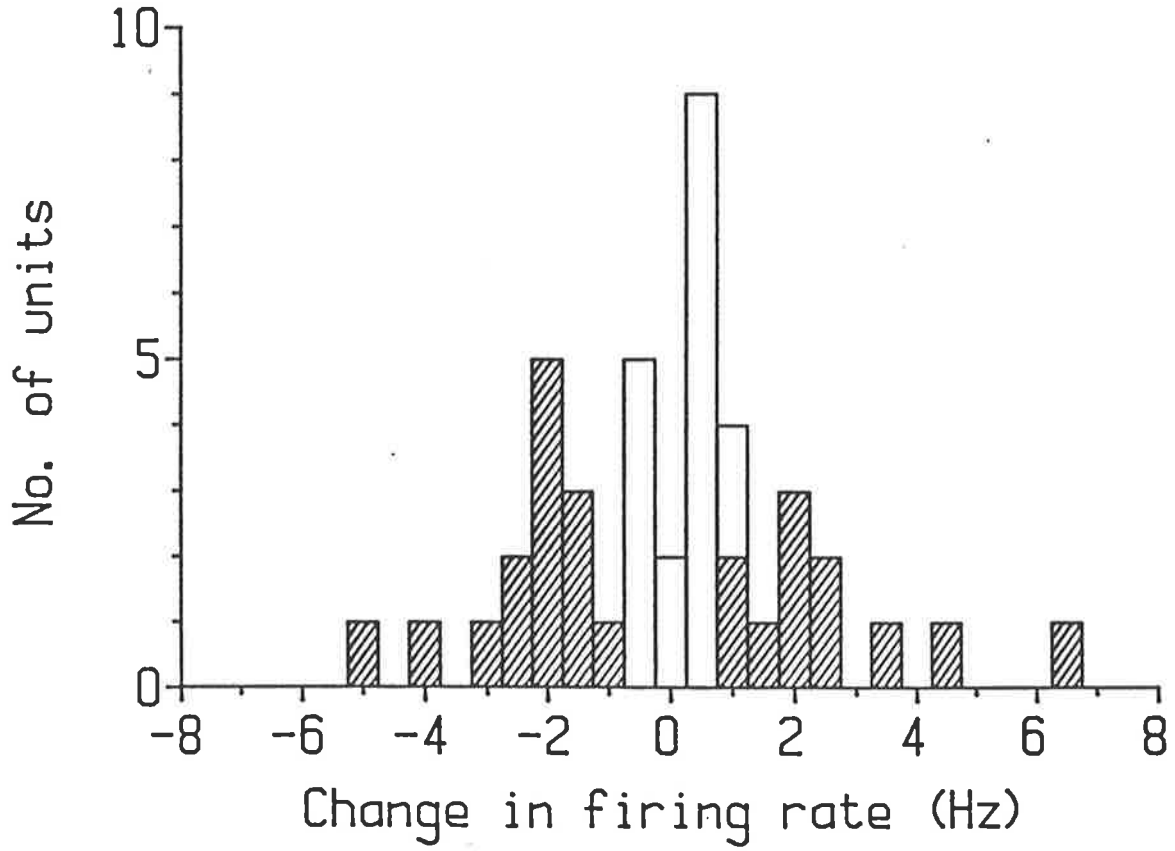

10
5
0
No. of units
-8
-6
-4
-2
0
2
4
6
8
Change in firing rate (Hz)

TABLE 9.1

DIRECTION OF CHANGE IN MEAN FIRING RATE FOR PAIRS OF BACKGROUND UNITS, IN EXPERIMENTS WHERE 2 OR MORE BACKGROUND UNITS WERE RECORDED IN ADDITION TO THE FEEDBACK UNIT.

		Background Unit 1			
		*	#	@	
	*	3	3	3	9
Background	#	1	1	3	5
Unit 2	@	3	3	4	10
		7	7	10	24

* firing rate did not change significantly after 15 minutes.

\# firing rate increased significantly after 15 minutes.

@ firing rate decreased significantly after 15 minutes.

An example of independent changes in mean firing rates of two background units is illustrated in Fig. 9.4. Unit *a* was the feedback unit controlled by the subject, and its mean firing rate remained constant at about 10 Hz for the duration of the test. Unit *b* progressively increased in rate from an initial 12 Hz to 14 Hz after 9 mins. Unit *c* decreased in rate from 17 Hz to 15 Hz over the same interval. The changes in mean ISI of units *b* and *c* were statistically significant ($P < 0.01$).

The mean firing rate of the 43 background motor units in the first minute of the contraction ranged from 7.3 – 20.8 Hz. A histogram showing the distribution of initial mean firing rate of the background units' included in this study is shown in Fig. 9.5*A*. Most background units were firing considerably faster than the feedback unit; only about 14% of background units fired at 10 Hz or less, while 61% had firing rates above 13 Hz. The relationship between the background unit's initial mean firing rate and its subsequent change in rate after 15 minutes is shown in Fig. 9.5*B*. The values for the units which "dropped out" before the end of the contraction, but which could be reactivated later by a harder bite, are indicated by the open circles in Fig. 9.5*B*. For the population as a whole, linear

FIGURE 9.4

Three concurrently – active motor units showing divergent changes in mean firing rate with time. The mean firing rate of the feedback unit (trace *a*) was controlled at 10 Hz by the subject. During this contraction the mean firing rate of one background unit increased (trace *b*), while the mean firing rate of another background unit decreased (trace *c*). The mean firing rates of units *b* and *c* at the end of the contraction were significantly different from their initial values ($P < 0.01$).

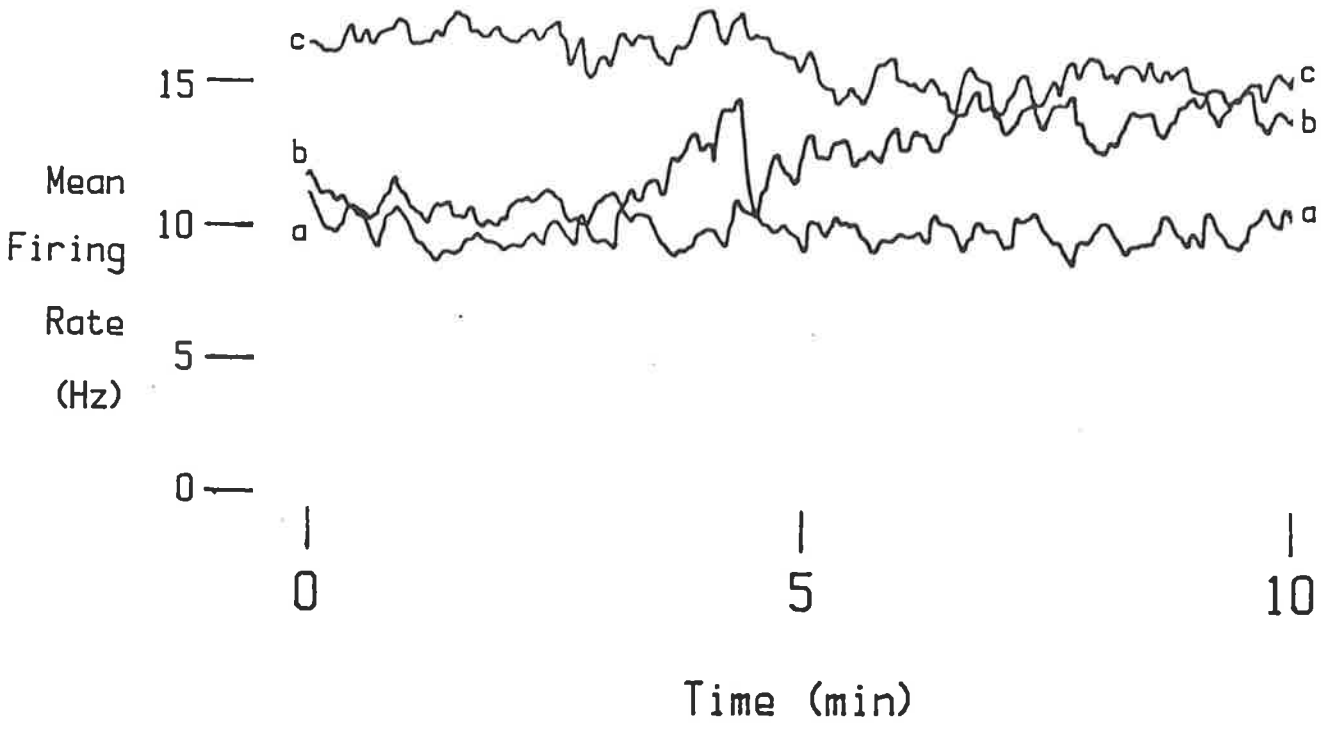
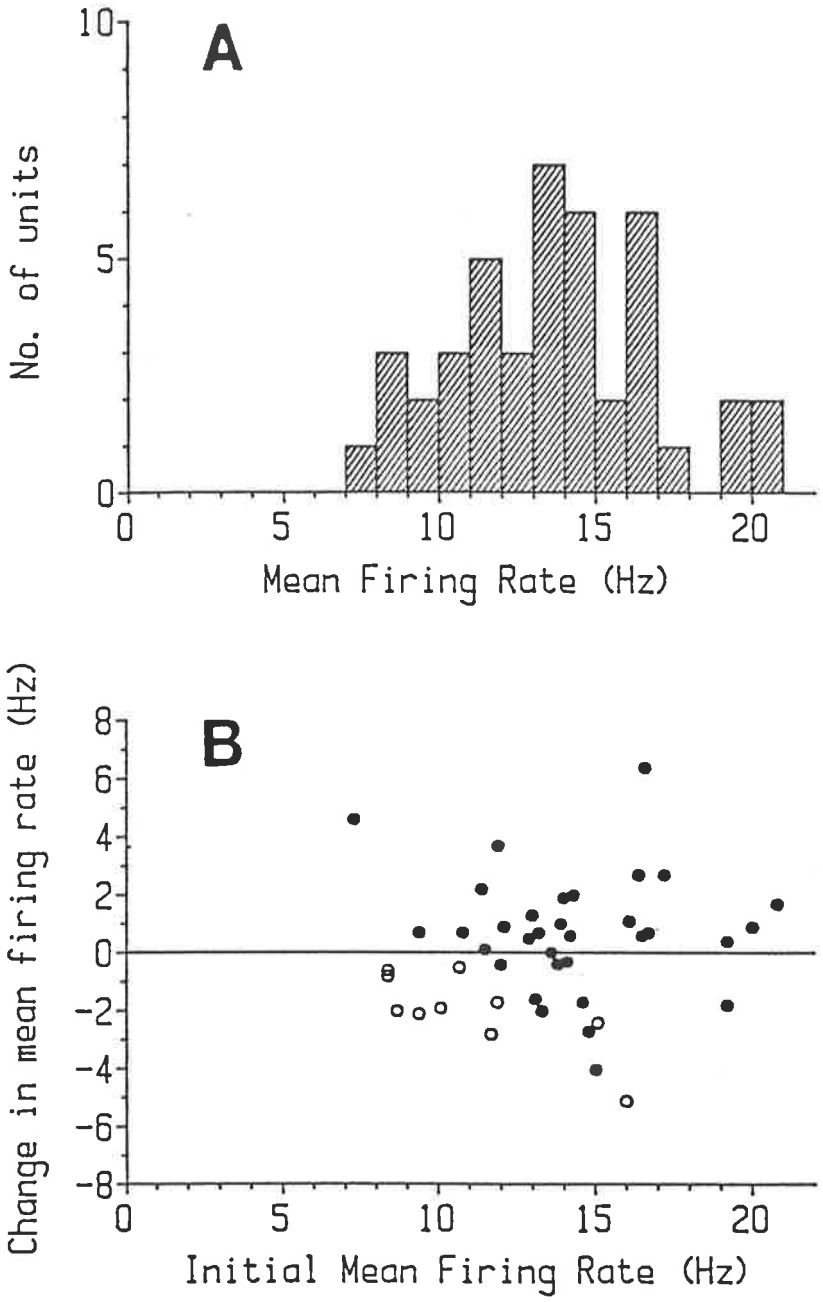

c
15
b
Mean
Firing
Rate
(Hz)
10
a
5
0
c
b
a
0
5
10
Time (min)

FIGURE 9.5

The initial mean firing rate of the background units, and its influence on the change in mean rate with time. *A*: Histogram showing the distribution of the initial mean firing rate of the background units. *B*: The relationship between the initial mean firing rate of the background unit, and its subsequent change in mean rate after 15 minutes of activity. The open circles represent the drop – out units.


A
No. of units
10
5
0
0
5
10
15
20
Mean Firing Rate (Hz)
B
Change in mean firing rate (Hz)
8
6
4
2
0
-2
-4
-6
-8
0
5
10
15
20
Initial Mean Firing Rate (Hz)

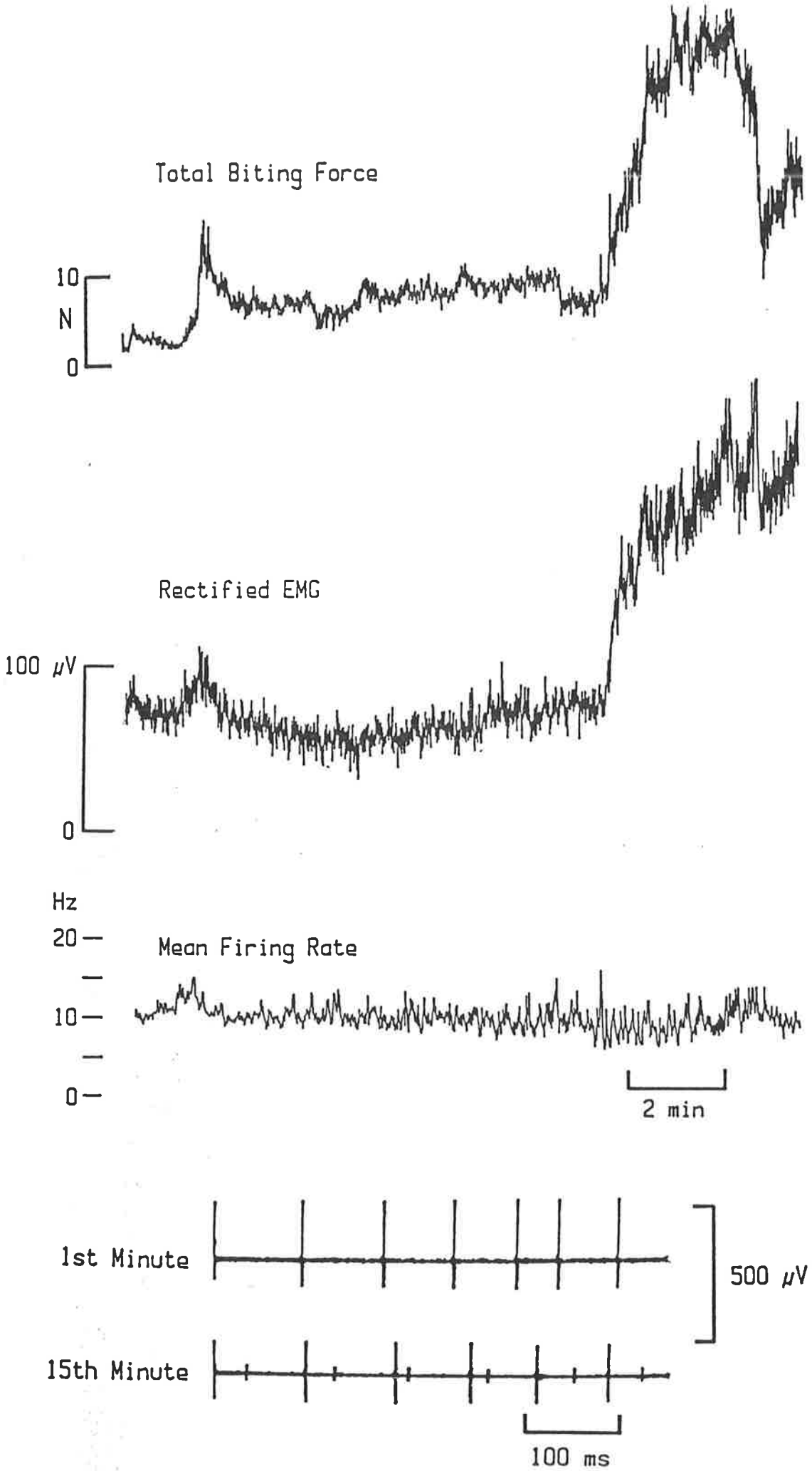
regression revealed no significant correlation between the initial firing rate and the change in rate over the 15 minutes ($r = 0.09$; $P > 0.05$). If the units which "dropped out" (open circles) were considered separately, there was a significant tendency for units with the higher initial mean firing rate to show a greater reduction in firing rate prior to drop – out ($r = -0.76$; $P < 0.05$); this is not surprising as most units ceased firing tonically at about the same mean rate (around 7 Hz).

The preceding results show that during a continuous isometric contraction all masseter units do not maintain a constant relative firing rate when the firing rate of one unit is kept constant. In some cases, the change in rate of the background units was quite large, which means that some motor units within the masseter pool are capable of strongly divergent firing behaviour during a continuous isometric contraction. On some occasions, the feedback unit was the one to display a firing pattern that was markedly divergent from the majority of the other active units. The evidence for this conclusion was found in the pattern of changes in the masseter surface EMG and biting force records. Fig. 9.6 demonstrates an extreme example. In this case only one intramuscular electrode had been inserted into the masseter, and the feedback unit was controlled at 10 Hz during a fatigue test. The lowermost traces in Fig. 9.6 show segments of the intramuscular EMG record. At the start of the contraction, the feedback unit was the only active unit detected on the electrode. By the end of the contraction the feedback unit's action potential had decreased in amplitude, but this had been observed to occur slowly and progressively, and three independent observers were confident of the identity of the unit. The activity of a second unit also became evident by the end of the contraction; this may indicate recruitment, or may simply be due to electrode movement. The trace of the mean firing rate of the feedback unit with time shows that the rate was maintained around 10 Hz for the duration of the contraction. In contrast, both the force and masseter EMG activity showed a marked increase after about 13 minutes. The total electrical activity of the masseter more than doubled in this time, and the recruitment threshold of the feedback unit (using the criteria of Chapter 7) increased from 3 N to 33 N. The only possible explanation for this result is that while the feedback unit was maintained at a constant firing rate, the net excitation (number of units active and/or their firing rates) of the masseter motor unit pool as a whole increased considerably.

During the contractions in which one unit in the masseter was controlled at a mean rate of 10 Hz for 15 minutes, the changes in the total biting force and

FIGURE 9.6

Atypical force and EMG records for one feedback unit. The two lower traces show segments of the intramuscular EMG record from the first and 15th minute of the contraction. The feedback unit was unambiguously identifiable throughout, although by the 15th minute its action potential amplitude had decreased, and the activity of another unit could also be detected on the electrode. The mean firing rate was maintained at 10 Hz for the duration of the contraction. The trace shown is the instantaneous firing rate smoothed by a sliding window average of the previous 16 ISI's. The two upper traces are the total biting force and rectified masseter EMG. The initial biting force was low, with a sharp increase at about 2 minutes, and then plateaued until a large increase at about 12 minutes which was maintained for two minutes before a reduction in the last minute of activity. Parallel changes are seen in the rectified EMG trace from the masseter muscle.


Total Biting Force
10
N
0
Rectified EMG
100 μV
0
Hz
20
10
0
Mean Firing Rate
2 min
1st Minute
15th Minute
500 μV
100 ms

electrical activity in the masseter were not always consistent. The most common pattern was for the total biting force to decline gradually with time, with little change in the masseter IEMG. Two examples of this are shown in Fig. 9.7. This result is consistent with that expected from contractile fatigue in the masseter units, and the maintenance of approximately constant excitation of the muscle. However, although this was the most common pattern, it was not the only one observed. Virtually any combination of force and EMG change was possible. Fig. 9.8 shows three examples; in each case the feedback unit was readily discriminated without ambiguity, and controlled at 10 Hz by the subject. In *A*, both force and masseter surface EMG activity declined in parallel, suggesting that force loss may be secondary to a reduction in the electrical activation of the muscle. However, the examples in *B* and *C* show that other EMG/force relationships were possible. In *B*, the masseter EMG activity declined considerably over the 15 minutes, yet the force dropped only in the first minute, and then stabilized. The record in *C* was even more unusual, in that the masseter EMG remained relatively constant while the biting force increased dramatically over the 15 minutes. The discrepancy between the force and EMG behaviour presumably results from a shift in the relative contribution of the masseter muscle to the jaw-closing force during some contractions. Therefore, the relationship between masseter surface EMG activity and biting force during these experiments was not consistent, and the value of one could not be used to predict the other reliably.

9.4 Discussion.

The main finding in the present analysis was that the mean firing rates of all motor units in the human masseter were not stable with time while the mean firing rate of one unit in the muscle was kept constant voluntarily by the subject. Only 42% of background units maintained a constant mean firing rate over the 15 minutes. A change of rate of 1 Hz was in most cases sufficient for a statistically significant difference between the initial and final mean firing rates, regardless of the actual value of the initial rate (Fig. 9.3). In some cases the differential changes were large, and sufficient to reverse the original rank order of motoneurone firing rates (e.g., Fig. 9.2), or markedly alter the force threshold of activation of the feedback unit (e.g., Fig. 9.6).

The initial mean firing rates of the background units were distributed over a large range, which emphasises the importance of firing rate modulation for force

FIGURE 9.7

The most common pattern of change in total biting force and rectified masseter EMG during the experiments. *A* and *B* show the changes in total force and rectified masseter EMG in separate experiments in which a feedback unit was controlled at 10 Hz for 15 minutes. The traces illustrate the most common pattern of change in these experiments, which was a slow decrease in the total biting force with little change in the masseter surface EMG activity.

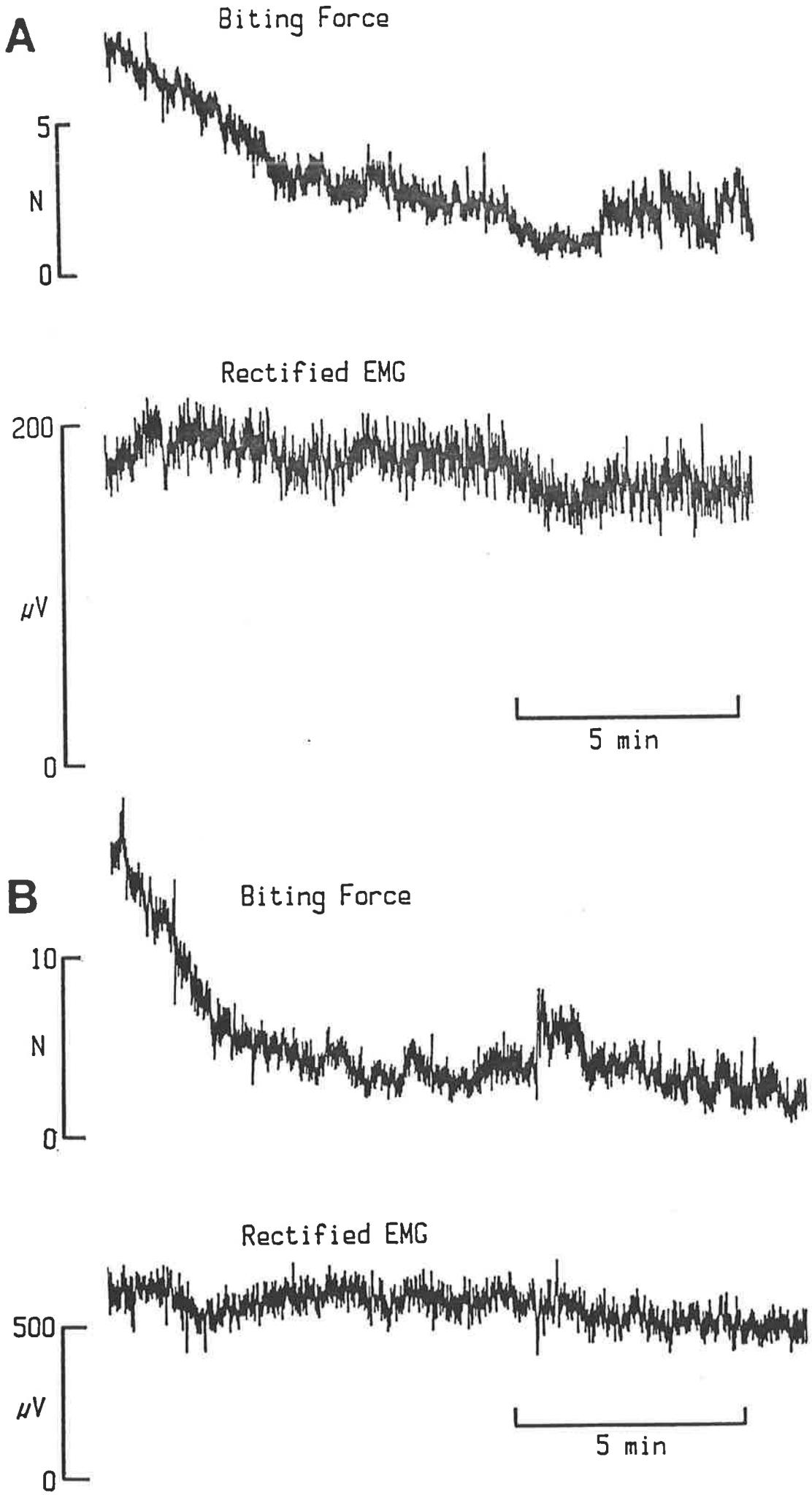
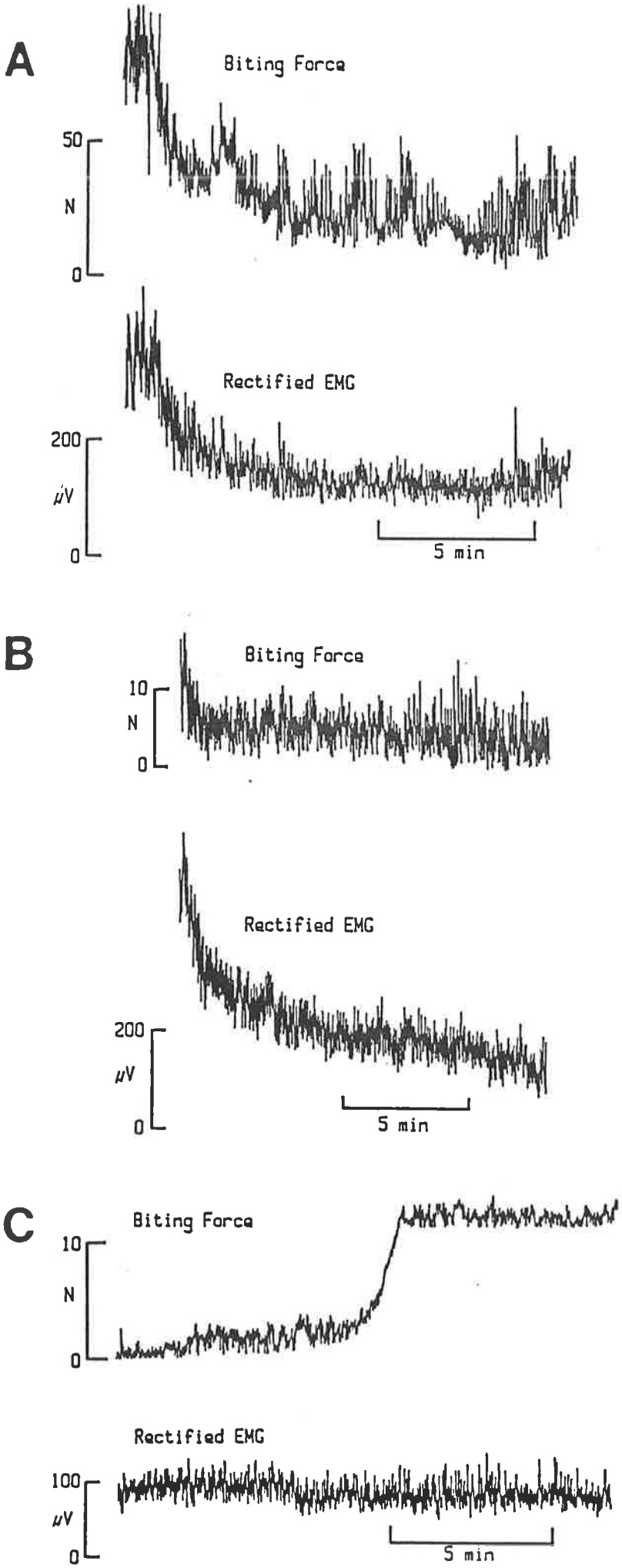

A
Biting Force
5
N
0
Rectified EMG
200
μV
0
5 min
B
Biting Force
10
N
0
Rectified EMG
500
μV
0
5 min

FIGURE 9.8

Three examples of biting force and rectified EMG changes which did not conform to the usual pattern. The biting force and rectified masseter EMG records from three different experiments are shown in *A*, *B* & *C*. In each case the feedback unit was controlled at a mean rate of 10 Hz throughout the contraction. Note the different time scale in *B*.


A
Biting Force
50
N
0
Rectified EMG
200
μV
0
5 min
B
Biting Force
10
N
0
Rectified EMG
200
μV
0
5 min
C
Biting Force
10
N
0
Rectified EMG
100
μV
0
5 min

production in the masseter. Fig. 9.5*A* shows the distribution of initial firing rates for the background units included in this study. The actual distribution of firing rates for active masseter units would be skewed towards even higher rates, but many small, high – rate units could not be discriminated with sufficient accuracy for inclusion in the present study. Many of these registered mean firing rates above 15 Hz in spite of the fact that an unacceptable proportion of their action potentials were not recognized due to superimposition with potentials from larger units.

The relative firing rates of a pair of units in short – term contractions reflects their recruitment order and hence their relative size (Tanji & Kato, 1973*b*). Units recruited at lower force levels have the higher mean firing rate at any level of total force. This has been verified for masseter motor units (Goldberg & Derfler, 1977; Miles & Türker, 1987). Therefore, background units with an initial firing rate above 10 Hz can be assumed to be smaller than the feedback unit, and background units with an initial firing rate less than 10 Hz can be assumed to be larger than the feedback unit. The change in firing rate in the background units with time did not correlate with the unit's initial firing rate (Fig. 9.5*B*). This means that the factors underlying the relative change in net excitation of the feedback and background units was not influenced by, or organized systematically in accordance with, the size of the motor unit. In some cases the changes accentuated the size – related differences in firing rate of some unit pairs, yet in other pairs the changes reduced the size – related differences in mean firing rate, even to the extent of reversing the original size – determined rank order of firing rates. The present results show that, following contractions of long duration in the masseter, the recruitment order cannot be inferred from the relative firing rates of the motor units. Furthermore, the approach of controlling the mean firing rate of one unit in the muscle during a prolonged contraction did not ensure constant excitation of the whole muscle. This would have been so if the units had maintained their relative firing rates throughout, and no recruitment of new units occurred. If this had been the case, it would have provided a simple means of controlling the net excitation of the whole muscle, for example during submaximal fatigue testing, where changes in the surface EMG signal occurring concurrently with fatigue make the surface EMG an unreliable index of whole – muscle excitation. Clearly, the approach of maintaining the masseter surface EMG at a constant level in the present experiments would not have ensured constant excitation of the muscle (i.e., the same motor units active at the same firing rates).

In the present study, rather than use total force or the EMG signal to control the level of excitation of the muscle, the mean firing rate of one unit was controlled, and firing rate changes of other concurrently – active units were used to indicate changes in the relative excitation of the units. By controlling the mean firing rate of one unit at a constant value, one is effectively controlling the net excitatory drive of that motoneurone. This is comprised of the descending voluntary drive from higher centres, together with the effects of reflex afferent EPSPs and IPSPs acting on the motoneurone. In addition, biophysical properties related to the motoneurone itself (e.g. adaptation) influence the output. Therefore, a change in the firing rate of one motor unit with time while the firing rate of another is controlled, as was frequently observed in the present study, indicates a differential change has occurred in one or more of the three properties mentioned above that determine motoneurone firing rate. These properties also control the recruitment threshold or functional grade of the unit. Each of these possibilities will be examined below:

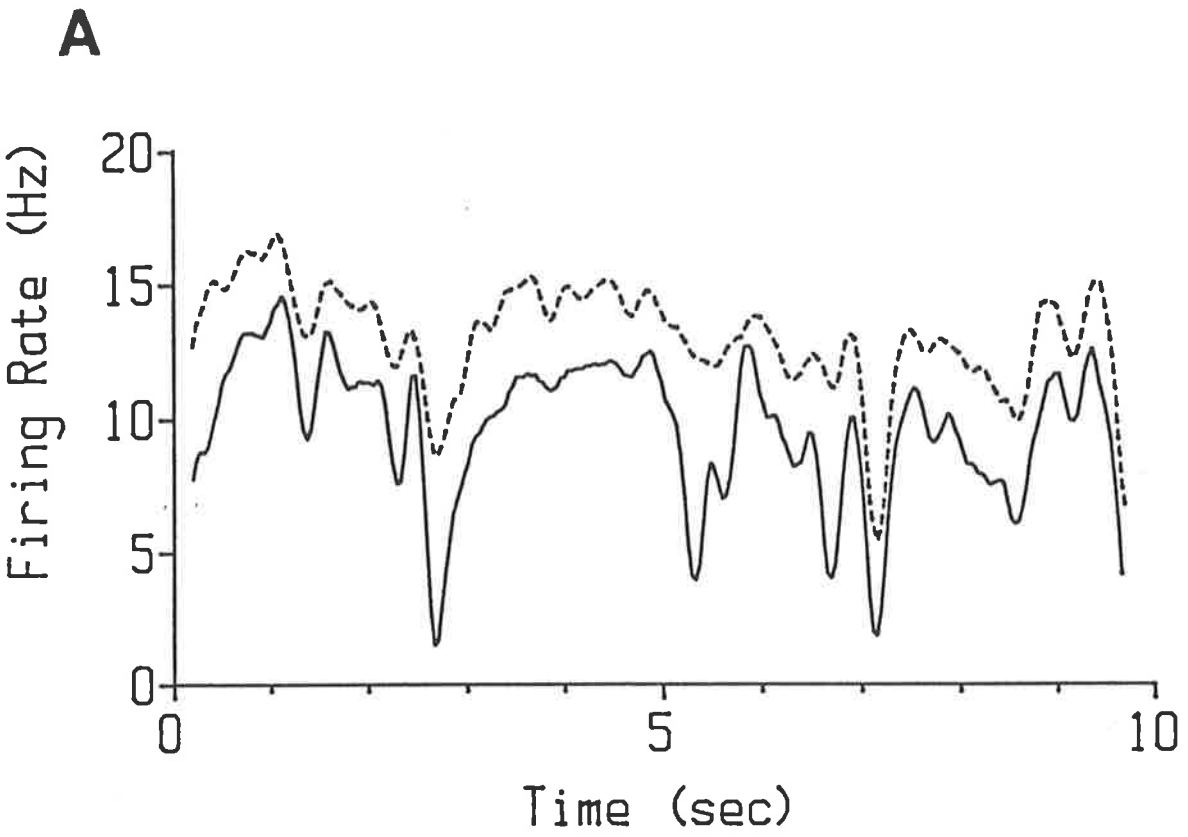
Possibility A: A differential central excitatory drive to the motor units.

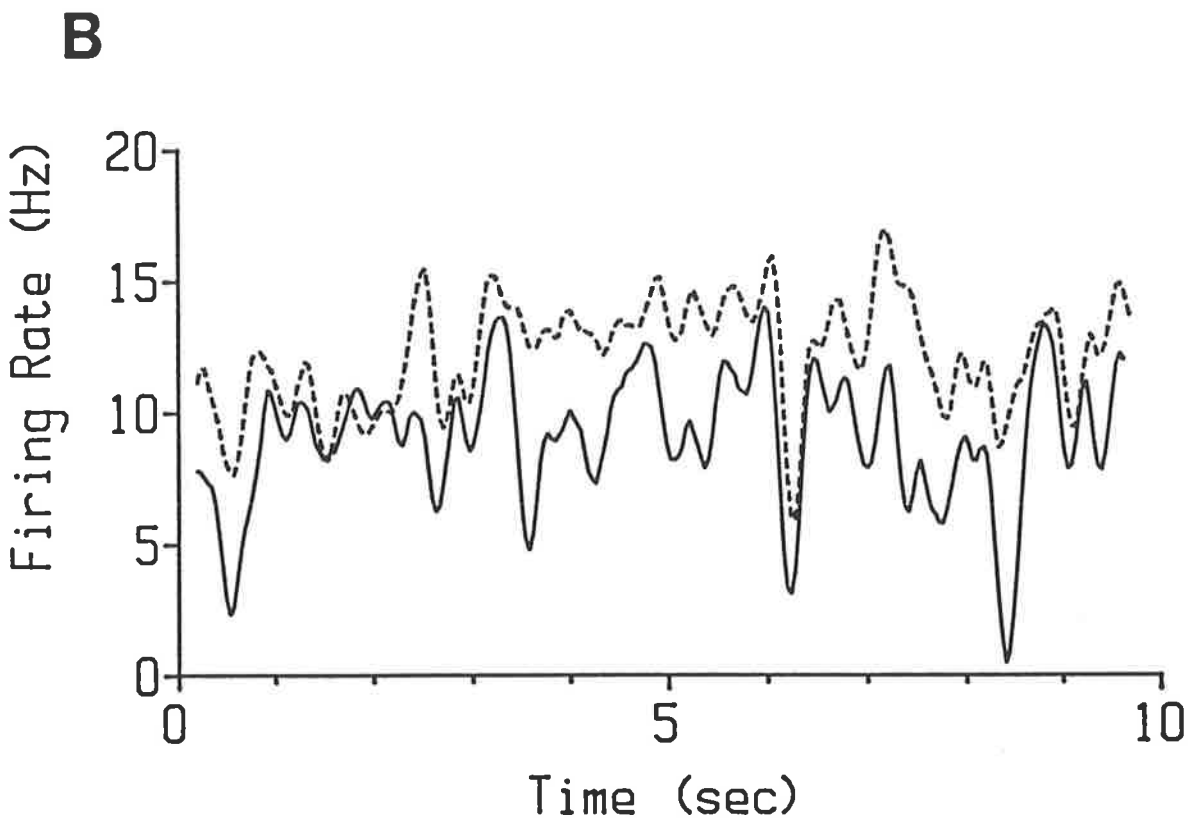
The non – uniform, long – term firing rate behaviour of the masseter units was unlikely to be the result of a differential central excitatory drive to the motoneurones. There is a great deal of evidence which suggests that the activity of motor units during the production of force is inter – related, so that truly independent voluntary control of motor units is not possible. Features such as the existence of a size – related recruitment order, and a roughly proportional increase in firing rate of active units in the muscle as the force is increased (Monster & Chan, 1977; Person & Kudina, 1972; Milner – Brown *et al.*, 1973*c*), together with the simultaneous common modulation of motor unit firing rates during attempted constant – force contractions in limb muscles (De Luca *et al.*, 1982*b*), and short – term synchronization (Chapter 2) all support the notion that motoneurones receive a widely – distributed, uniform, command signal with a large number of common inputs.

An incidental finding in the present study was the demonstration of the existence of a strong common modulation of firing rate ("common drive") in the masseter motor units (Fig. 9.9). The fluctuations in the filtered firing rates of masseter unit pairs were highly correlated, despite the fact that the subject was attempting to keep the mean firing rate of one unit constant. The trigeminal system has several unique features which make this an interesting finding. Unlike the limb muscles, the masticatory muscles lack recurrent inhibition (Lorente de Nó, 1947;

FIGURE 9.9

"Common Drive" in masseter motor units. The smoothed firing rate records for two pairs of motor units from different subjects are plotted against time in *A* and *B*. In each case the filtered firing rate of the feedback unit is the solid line, and that of a concurrently – active background unit is represented by the dashed line. The instantaneous firing rate was digitally smoothed using a Hanning sliding window filter (400 ms wide). In both *A* and *B* there is a strong correlation between the peaks and troughs in the solid and dashed firing rate traces, which result from common fluctuations in the net excitatory drive to the motoneurones.


A
Firing Rate (Hz)
20
15
10
5
0
0
5
10
Time (sec)


B
Firing Rate (Hz)
20
15
10
5
0
0
5
10
Time (sec)

Shigenaga *et al.*, 1988), and probably also Golgi tendon organs (Luschei & Goldberg, 1981), and the jaw-elevator muscle spindle afferents do not project uniformly to all motoneurones within the parent muscle (Appenteng *et al.*, 1978). Therefore, inputs from these sources do not appear to be important determinants of "common drive", which suggests that it arises principally from common descending inputs.

Possibility B: A differential change in motoneurone biophysical properties with time.

One possible explanation for the present results is a differential adaptation of the motoneurones to excitatory currents. Motoneurones in the cat can be made to fire repetitively by current injection via an intracellular microelectrode, as a substitute for the steady post-synaptic currents that would normally excite them during prolonged activation *in vivo*. It has been shown that these motoneurones are subject to adaptation so that the injection of a constant current results in a fall in the firing rate with time. In the experiments of Kernell and Monster (1982*a*,*b*), adaptation with a slow time course (tens of seconds) was observed, and termed by them "late" adaptation. The firing rates reached a steady state after about 1 minute and no further adaptation was seen for repetitive discharges lasting up to 4 minutes. The amount of late adaptation was also found to be linearly related to the initial firing rate of the motoneurone, with the faster-firing units showing the greatest adaptation (Kernell & Monster 1982*a*).

The changes in firing rate observed in the present study did not fit the pattern of late adaptation described by Kernell & Monster. The changes in rate occurred with a slow time-course, and frequently occurred after several minutes of repetitive firing (e.g., Figs. 9.2, 9.4). Also, the change in firing rate in the background units was not influenced by their initial firing rate (Fig. 9.5). It would be impossible for the type of adaptation described by Kernell & Monster to produce a result in which a unit with an initial faster firing rate increased its rate with time, while a unit firing at a slower rate maintained a constant rate, because this form of adaptation would tend to bring their relative firing rates closer together. However, this pattern of behaviour was seen on a number of occasions in the present study (Fig. 9.5*B*). Finally, cat motoneurones showed no adaptation at all when the initial firing rate was 10 Hz or less (Kernell & Monster, 1982*b*), yet a masseter unit with an initial firing rate of around 8 Hz was observed to increase its firing rate slowly by 5 Hz while the feedback unit was controlled at 10 Hz (Fig. 9.2).

Although the late adaptation described by Kernell & Monster does not fit the present experimental evidence, some other form of motoneuronal adaptation cannot be entirely ruled out. There is no experimental data on the effects of repetitive discharge for periods approaching 15 minutes because of technical limitations of the current injection techniques, so other adaptive properties may exist of which we are not aware. It is possible that the time – dependent changes in ISI variability described in the previous Chapter reflect a form of adaptive change in the motoneurone, but further experimental work is required to test this hypothesis.

Possibility C: A differential change in the balance of the reflex afferent excitatory and inhibitory influences with time.

Reflex afferent influences which are unequally distributed amongst the motoneurones in the pool could mediate the observed differential changes in motor unit firing rates. One such peripheral system is the Renshaw cell recurrent inhibition, which has been implicated as a possible explanation for the non – uniform changes in firing rates in tibialis anterior motor units associated with the recruitment of a new unit during slow, isometric force – ramps (Broman *et al.*, 1985). However, the motoneurones of the jaw muscles are considered to lack Renshaw – type recurrent inhibition (Lorente de Nó, 1947; Shigenaga *et al.*, 1988), so these circuits are unlikely to be responsible for the present results.

In the limb muscles, muscle spindle afferents project widely to influence nearly all motoneurones within the pool on which they act (Henneman & Mendell, 1981). There is evidence that this is not the case for jaw elevator muscles in cats, in which it was concluded that spindle afferents project to only a small proportion of homonymous motoneurones (Appenteng *et al.*, 1978). Therefore, muscle spindle afferent connexions in the masseter have the potential to produce differential effects on motoneurones. A possible "reflex partitioning" of the masseter has not been systematically studied in animals. The changes in the present study were not size – structured, nor was there any suggestion that they were location – specific; these two features have been suggested to be the most likely benefits of a functional reflex partitioning of muscle spindle afferent influences.

Cutaneous afferent stimulation can alter the recruitment order of motor units in the first dorsal interosseous (FDI) muscle in humans (Garnett and Stephens, 1981). This implies that these afferents have a differential input to the motoneurones of different sizes. A time – dependent change in the activity of these receptors would therefore be expected to have similar non – uniform effects

on tonically – active motor units. It is probable that the output of receptors in the gingiva and periodontal ligament changed with time during the present experiments. It was common for the subjects to feel progressive parasthesia and even anaesthesia in the tissues supporting the teeth during a continuous bite for 15 minutes, particularly during forceful bites. The receptors in the oral structures could mediate differential reflex effects on the motor units if the distribution or effectiveness of their synaptic connexions to masseter motoneurones were not uniform, and also not size – structured. There is at present little evidence on the weighting of the connections of oral afferents to jaw elevator motoneurones, although Miles and Türker (1986) concluded that the Aδ fibres innervating the lip exert a similar influence to most or all masseter motoneurones.

Regardless of the source of the differential changes in motor unit activity with prolonged activation, these results demonstrate that motor unit firing patterns are not static, but may be modified during a continuous isometric contraction. Although they were comparatively rare, large deviations from the original rank order were found in some units with prolonged activity. The finding that masseter motor units may be selectively suppressed or facilitated during a long contraction lends support to the old notion of rotation of motor unit activity. The functional significance (if any) of these observations remains to be elucidated. It is conceivable, for example, that motor unit activity during a prolonged contraction may be modified either reflexly, or because of some biophysical property of the motoneurone, so as to minimise the effects of fatigue without the need for any central processing.

CHAPTER 10

CONCLUDING REMARKS

This thesis has been concerned with the functional characteristics of human masseter motor units. The main aim of this series of experiments was to quantify fatigue in masseter units, in order to provide a more complete understanding of their physiological properties, which would aid the interpretation of the peculiar histological appearance of the masseter. The human masseter was found to be comprised predominantly of fast – twitch motor units with a broad spectrum of fatiguability. Very few physiological type S units were found, despite histochemical evidence for a substantial population of type I fibres in the masseter. A substantial number of fast – twitch, fatigue – resistant motor units (type FR) were found in the masseter, yet the human masseter generally lacks the histochemical type IIA fibres. It is suggested that some physiological type FR motor units in the masseter have muscle fibres which stain as histochemical type I. The correlation between motor unit physiological and histochemical properties is apparently not as strong in the human masseter as is generally found in other muscles. Most of the studies in which direct comparisons have been made between motor unit physiological and histochemical/biochemical properties have involved the cat hindlimb muscles, and it may be unwise to generalize these relationships to all muscles and species. In order to clarify this issue, it is suggested that direct comparisons employing glycogen depletion techniques, preferably with quantitative microanalytical biochemistry (e.g., Hamm *et al.*, 1988), are required in several different human or primate muscles.

The masseter was also unusual in that no significant correlations were found between fatiguability and either twitch amplitude or contractile speed in the motor units studied. These findings are not so surprising when they are evaluated together with the muscle's functional requirements, and the available histological data, but they illustrate once again the danger of generalization.

Despite these peculiarities of masseter motor units, comparison with other human fatigue data suggests that as a group, masseter motor units active during low – and moderate – force contractions have a similar distribution of fatiguability after approximately 3000 activations as units in human first dorsal interosseous and medial gastrocnemius.

The contribution of individual motor units to the surface EMG was not constant with time. The usual finding was a progressive change in amplitude of the unit

surface representation waveform which was correlated with the size of the motor unit: small units tended to increase in amplitude and large units tended to decrease in amplitude. In contrast to the large changes in amplitude of the motor unit surface potentials, the duration of the waveform was quite stable. The explanation for these findings remains unclear at present. However, they highlight the complexity of the surface EMG signal, and suggest that the surface EMG signal cannot be relied upon to give an accurate indication of muscle excitation during prolonged contractions, since the contribution of each unit to the signal is not constant with time.

There were no significant correlations between the changes in the motor unit's electrical signal and contractile fatigue during these experiments. Under these conditions, fatigue was not due to activation failure. Since contractile fatigue was objectively measured in this study, and changes in the EMG signal were not related to the contractile state of the motor unit, it is concluded that some fatigue processes cannot be detected by analysis of the surface EMG signal alone.

The spike – triggered averaging (STA) technique was thoroughly evaluated, and found to be suitable for use in the masseter. The need to assess motor unit synchronization accurately led to the use of cross – correlation interval histograms, with which masseter motor units were found to exhibit weak, but widespread synchrony. It was concluded that this synchrony did not invalidate the use of STA in the masseter. The synchronization study in itself had a wider significance. The high proportion of masseter units with a tendency to synchronous firing indicates that many masseter motoneurones have common synaptic inputs. The use of a test of significance for synchronous peaks in the cross – correlograms demonstrated the importance of collecting long periods of data in order to have sufficient counts to detect this weak synchrony, and it is suggested that the proportion of motor units with synchronous activity may have been underestimated in previous studies in other muscles because only short segments of data were analysed. It is generally believed that synchronization increases during prolonged, fatiguing contractions, but the surface EMG evidence from which this conclusion is drawn is not reliable. The present study is the first to objectively measure the effects of the duration of the contraction, and fatigue, on synchronization of motor unit pairs, and significant changes in synchronization were not found. Further studies using cross – correlation of unit firing times and a variety of fatiguing paradigms are indicated to clarify this issue.

In another line of investigation into the STA technique, the immediate firing pattern of the trigger spike was shown to have a considerable influence on twitch measurement with STA. It was necessary to select valid trigger spikes in the fatigue studies on the basis of their pre – and post – trigger firing intervals in order to minimise errors in the STA twitch from fusion, and the correlated activity of other motor units in the muscle. Although the spike parameters chosen for the fatigue study resulted in some fusion of the twitches, this did not distort the fatigue measurements. It is undoubtedly fortunate that the masseter is predominantly comprised of fast – twitch motor units; in other muscles with generally slower – twitch motor units, the deleterious effects of partial fusion on the STA twitch are likely to be greater.

Several aspects of the control of motor units were also studied. The activation force threshold of the units was found to vary systematically with muscle length. It was concluded that the changes were a consequence of the length – passive tension relationship of the muscles, joint and soft tissues. In addition, the motor unit action potential recorded with a fine – wire intramuscular electrode was also found to vary with alterations in muscle length; probably as a result of changes in the geometric relationship of the electrode to the muscle fibres at various muscle lengths. These results indicate that attempts to assign masseter units to one or other of the accepted functional categories on the basis of recruitment force or waveform only are unlikely to give reliable results.

The regularity of discharge of masseter motor units was studied, and correlations were sought with physiological properties of the motor units. A significant correlation was found between motor unit fatiguability and its firing regularity. This is a previously unrecognized link between the motoneurone and the muscle fibres innervated by it.

A model of the effects of noise in the motoneurone membrane potential trajectory on interspike interval (ISI) variability was developed, based on evidence from studies of rhythmic firing mechanisms in cat motoneurones. This model explains the observed relationship between mean ISI and discharge variability in human motor units, without the need to postulate changes in the properties of the synaptic noise. It provides circumstantial evidence that spike – generating mechanisms are similar in human motoneurones responding to voluntary drive to those found in cat motoneurones driven by intracellular current injection. This assertion can be tested directly by a new approach to reflex testing which has been developed in this laboratory, and will be the subject of future experiments.

During a prolonged isometric contraction in which the firing rate of one unit was controlled voluntarily by the subject at a constant level, the mean firing rates of other masseter units were not stable. In some cases, large changes in rate were seen, which were sufficient to reverse the original rank order. The independent behaviour of the motor units was not size – related. The most likely explanation for these findings is a differential change in the reflex afferent input to the motoneurones, although differences in motoneuronal adaptation cannot be excluded. The results indicate that the combination of motor units used to perform a particular task is not rigidly fixed by the motor units' size, but may be modified during a continuous isometric contraction.

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APPENDIX A

Assessment of the statistical significance of peaks in cross – correlation histograms (after Wiegner & Wierzbicka, 1987).

A problem with interpretation of cross – correlation histograms has been the assessment of the significance of peaks in the histogram. For a peak to be significant, the excess counts within the peak must be greater than the number expected by chance. In order to assess this, the counts within the peak must be compared to the mean bincount over the non – peak part of the histogram and the variance of the bincounts. The method of Wiegner & Wierzbicka (1987) was used for this purpose in the present study, and the calculations are described below.

Some Definitions:

a) the total number of counts (T).

b) the total number of bins (N).

c) the number of bins in the peak to be analysed (J).

d) the mean bincount in the non – peak (N – J) bins ($\underline{b}$). If $\underline{b} < 9$, the histogram was not analysed further.

For all histograms in the present study; N = 201, J = 7 & binwidth was 1 ms. A Synchronization Index (SI) was calculated for each histogram as follows:

$$SI = [\text{counts within the J bins} - \underline{b}] / T.$$

To determine the statistical significance of this SI value it was compared to a critical value (CV) it would need to have for that histogram at 3 levels of significance ($P < 0.05$; $P < 0.01$; $P < 0.001$). The formula for CV is given by:

$$CV_\alpha = (Z_n / \text{sqr rt } NT) + (Z_\alpha \text{ x sqr rt } [J / (N-J)T])$$

The value used for Z_n for histograms with 201 bins was 2.81.
Z_α = 1.64 for $P < 0.05$, 2.33 for $P < 0.01$ & 3.09 for $P < 0.001$.
When an a priori decision was made to evaluate peaks around time 0, (Z_n / sgr rt NT) = 0, and was dropped from the equation. Peaks in histograms returning an SI greater than $CV_{0.05}$ were deemed to be significant ($P < 0.05$).

APPENDIX B

CURRICULUM VITAE

Personal

Name:	Michael Andrew NORDSTROM
Date of birth:	November 29th, 1957
Nationality:	Australian
Marital Status:	Married, no children
Present Position:	Dental Postgraduate Scholar, NH & MRC of Australia, Department of Physiology, University of Adelaide, GPO Box 498, Adelaide, South Australia 5000.

Academic Qualifications

Bachelor of Dental Surgery, University of Adelaide. Awarded May, 1980.
(Equivalent to DDS degree in the USA)

Awards

National Health and Medical Research Council of Australia Dental Postgraduate Scholarship, 1985 – .
Adelaide University Blue for cricket, 1979.
Represented Australian Combined Universities in cricket, 1979/80.

Appointments

Dental Surgeon with the South Australian Dental Service, 1980 – 84.
Private Dental Practice, 1984.
Part – time Dentist with the S.A. Dental Service 1985 – present.
NH & MRC Dental Postgraduate Scholar, 1985 – present.

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6. Nordstrom, M.A. & Miles, T.S. Synchronization of motor units in human masseter. In Preparation.

7. Nordstrom, M.A. & Miles, T.S. Changes in the contribution of single motor units to the surface electromyogram during a fatiguing voluntary contraction. In Preparation.

8. Nordstrom, M.A. & Miles, T.S. Observations on the variability of motor unit discharge in human masseter. In Preparation.

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APPENDIX C

Reprints of Published Papers associated with this Thesis (overleaf).

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J. Electrophysiol. Tech., Vol. 14, pp. 85–90, 1987
Printed in the USA.

0361-0209/87 $3.00 + .00

A CIRCUIT FOR IMPROVING THE PRECISION OF THE SPIKE-TRIGGERED AVERAGING TECHNIQUE

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Abstract—*The technique of spike-triggered averaging has been used to determine the isometric force developed in single twitches of individual motor units in man. This paper describes a simple circuit that will increase the time resolution of this technique by increasing the signal-to-noise ratio of the force signal being led into the averager or FM tape recorder. The circuit also increases the ease and accuracy of calibration of the force signal in the spike-triggered averaging technique.*

Keywords—Spike-triggered averaging, Motor unit twitch, Signal averaging

INTRODUCTION

The technique of ensemble averaging of event-related signals has been widely used in neurophysiology to extract a small signal from a noisy background. Two methods are commonly used to improve the resolution of the average. The first is to filter the signal entering the averager so that unwanted frequencies do not contribute to the averaging procedure. This technique is particularly useful when the bandwidth of the output signal is known in advance, although filtering may produce unwanted (and often undetected) distortions of the output waveform. The second method is to increase the number of trials that are averaged. The latter approach, while often useful, is not always practical, since the event being studied may itself change with time (e.g., a fatiguing muscle twitch) or the number of trials possible may be limited for technical reasons (e.g., the deterioration of a cell membrane potential during an intracellular recording or the limited time for which an extracellularly recorded action potential can be "held" due to electrode movement). In any event, the signal-to-noise ratio improves only in proportion to the square root of the number of trials and this is thus an approach of diminishing returns.

There are several experimental situations in which the desired signal is superimposed on a background potential whose amplitude fluctuates widely. This problem is compounded if the bandwidth of the background waveform is similar to the bandwidth of the desired signal. One example of this situation was encountered in this laboratory when the technique of spike-triggered averaging was used to determine the characteristics of the twitch produced by a single motor unit in a contracting human muscle. In

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this technique, an electrode is placed into a muscle to record the action potentials of a single motor unit. These potentials are then used to trigger an averager, with total muscle force as the input signal. Since each action potential in a slowly firing unit is followed by a twitch, averaging of the force signal will eventually yield the twitch.[1]

In this paradigm, the subject must voluntarily contract the muscle in such a way that the motor unit fires at a steady frequency of about 10 Hz; this is not difficult to achieve with the help of visual and auditory feedback of frequency. However, when one examines the total muscle force signal that is to be the input to the averager, two problems are immediately apparent. First, the force signal is usually offset considerably from ground potential, and second, the amplitude of the force signal fluctuates considerably even when the unit is running at an acceptably steady frequency. The consequence is that the twitch signal itself will occupy only a small fraction of the input voltage range of the averager.

Our initial attempt to resolve this problem was to high-pass filter the input signal to the averager using a filter with a time constant of 1.0 or 2.5 s as suggested earlier.[1] This had the desired effect of eliminating the offset potential, but the force fluctuations in which the twitch was "hidden" remained at an undesirably high amplitude, particularly with the 2.5 s time constant (Figure 3). Moreover, it was clear that the lower frequencies in the averaged twitch signal were close to the lower frequency limit of our filters; that is, the filters may have been distorting the actual twitch waveform.

A solution to this problem was suggested by Dr. Bruce Walsmley of the Australian National University. On his advice, we designed a sample-and-hold amplifier (SHA) that works in the following way. The input to the SHA is the raw force signal. Each action potential triggers the SHA to reset the force signal to near-ground potential. The part of the signal that is to be averaged occurs in the 100 ms or so after each action potential. Accordingly, if the input signal (force) is reset to ground potential by each action potential, the subsequent segment of the force signal containing the twitch can be led into the averager at a high amplitude relative to the input voltage range of the averager.

MATERIALS AND METHODS

Circuit Details and Operation

A block diagram of the circuit and its mode of operation is shown in Figure 1. The slowly fluctuating input signal [middle trace in Figure 1(B)] is offset some distance above ground potential. Each trigger pulse in the train (uppermost trace) forces the SHA output signal to near-ground potential (lowermost trace). From there, the output signal changes in parallel with the input signal until another trigger pulse resets it again to near-zero volts. This repositioned signal can then be amplified to a level appropriate to the input voltage range of the averager or it can be stored at high gain (i.e., with high signal-to-noise ratio) on FM tape for later off-line analysis.

The detailed circuit schematic is given in Figure 2. The trigger signal is an output pulse from an amplitude discriminator, which indicates the occurrence of each action potential in a motor unit. This trigger signal is conditioned and inverted by IC1 and IC2. The resulting negative pulse then triggers the sample-and-hold circuit, which consists of IC3, IC4, and IC5. IC3 is a collection of high-impedance gates, while IC4 and IC5 are high-impedance amplifiers. The trigger pulse causes the dc level of the data input signal (e.g., force) to be sampled: the input signal is then disconnected and the sampled voltage is held by the 0.01 μF capacitor. IC5 has a very high input impe-

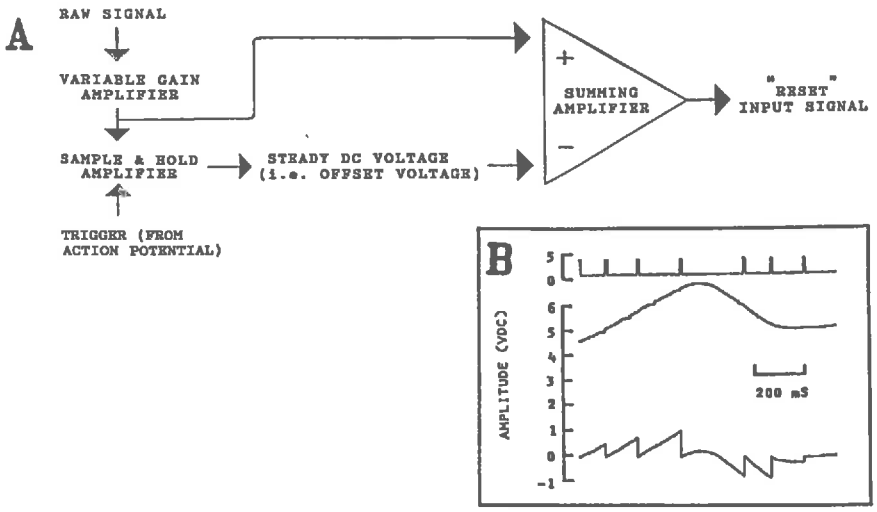

Figure 1. (A) Block diagram of the circuit. The modus operandi of the circuit is to subtract the dc offset in the total force signal at the time of the trigger pulse from the total force signal with the summing amplifier. (B) Operation of the SHA circuit. The analogue test signal (middle trace) has a dc offset of about 4.5 V. The dc component of this signal can be cancelled by the differential amplifier to near-zero volts on the occurrence of each of the trigger pulses in the uppermost trace: this produces the output shown in the lowermost trace. Note that the shape and amplitude of the output signal between successive pulses is unchanged from that of the input, but the output is now centered around ground potential (0 Vdc). This signal would now be led into an averager triggered by the pulses. The signals shown were recorded on and printed out by a digital oscilloscope; hence the small steps on the three traces.

dance, so its discharge time constant is long. The steady dc potential sampled at the time of the trigger pulse is then subtracted from the raw data signal by IC6 to give a net potential of near-zero volts; that is, the dc offset of the input signal is reset by the trigger pulse to near-zero volts without distortion of the subsequent part of the waveform. IC7 is a variable-gain amplifier with the gain potentiometer mounted on the front panel to enable the operator to adjust the amplitude of the SHA output to match the input voltage range of the averager or FM tape recorder. All components used are readily available for a total cost of less than US$50.

RESULTS

To test the effectiveness of the circuit, we averaged the same experimental force data in three different ways: (1) high-pass filtered at $\tau = 2.5$ s, (2) high-pass filtered at $\tau = 1$ s, and (3) with the SHA circuit. The gains of each of the three inputs to the averaging computer were adjusted so that the signal amplitudes did not exceed ± 1 Vdc input range throughout the 20 s averaging period (left side of Figure 3). Because of the slow fluctuations that remained in the force records after filtering, the maximal gain that could be used for these two inputs to the averager was 0.7 V/100 g. By comparison, when the SHA was used to remove the force fluctuations, the maximal input gain possible was 1.5 V/100 g.

To minimize summation effects, the averaging computer accepted for analysis only those spikes that were both preceded and followed by an interval of at least 100 ms in which no spikes occurred.

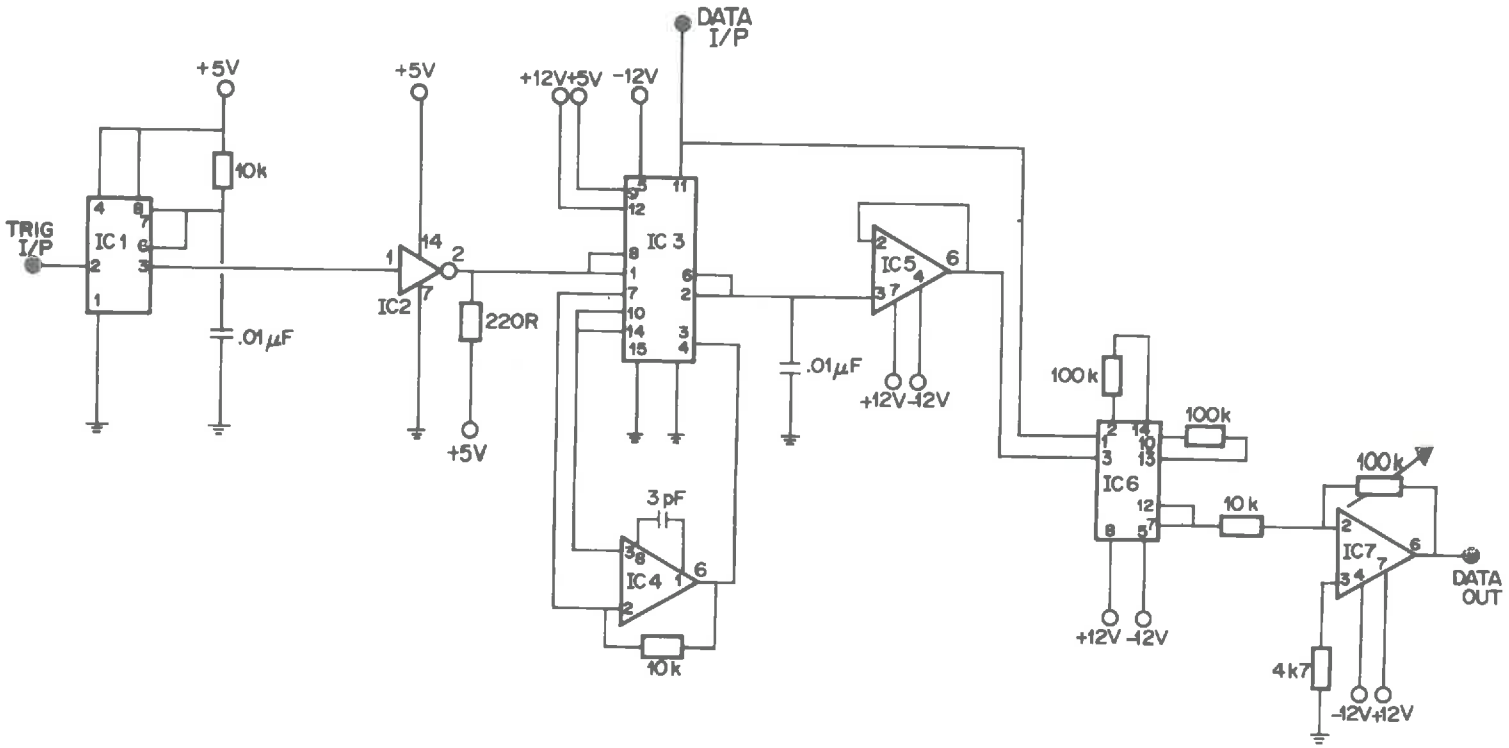

Figure 2. Circuit schematic for SHA device. IC1 555; IC2 7405; IC3 LF13331; IC4 and 5 LF 356; IC6 AD 521; IC7 LF 356.

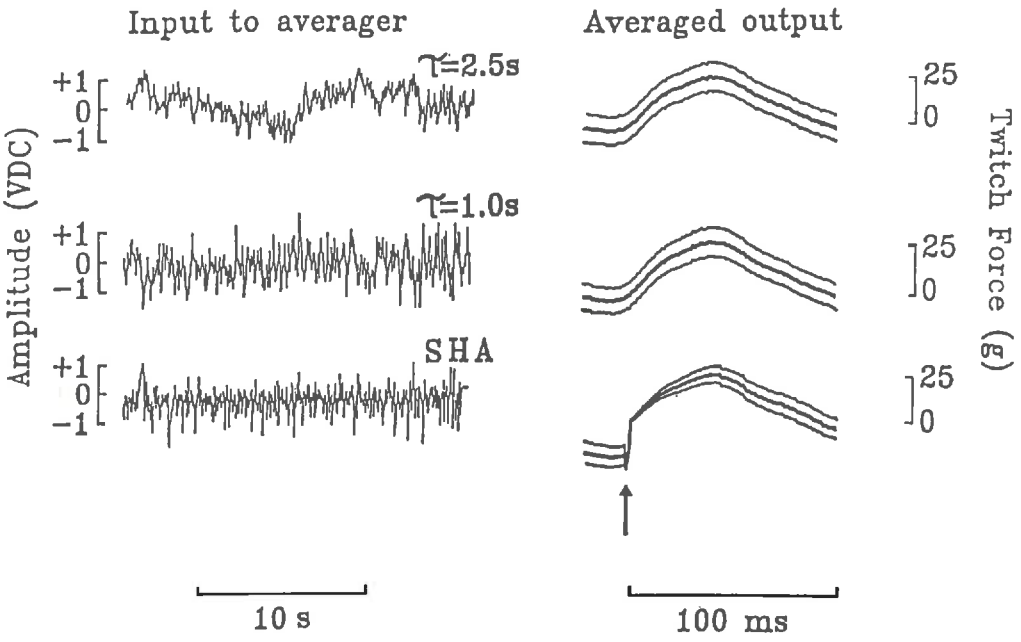

Figure 3. Comparison of the SHA approach with high-pass filtering when using spike-triggered averaging to measure single motor unit twitch force. The three traces on the left are the force input signals to the averaging computer; each one was adjusted to a ±1 V range. The upper trace is the result of high-pass filtering the force signal with a time constant $\tau = 2.5$ s, the middle trace is filtered at $\tau = 1.0$ s, and the lower trace is the output of the differential amplifier. The traces on the right are the computer-derived means ± standard errors of twitch force corresponding to the three different input signals. The timing of the motor unit action potential that triggered the averager is indicated by the vertical arrow. The 105 spikes selected by the computer for averaging were those that were preceded and followed by an interval of at least 100 ms in which no other spike occurred.

The result of averaging the same 20 s of data under the three different conditons is shown on the right-hand side of Figure 3. Each average (mean ± standard error) is plotted at the same gain. The peak amplitude of the twitch in both the SHA and the filtered records was 25 g in the example shown. However, at the peak of the twitch, the standard error of the mean for the filtered records was 9.2 g, compared to 4.6 g for the SHA.

DISCUSSION

The SHA circuit is designed to increase the precision of the spike-triggered averaging technique when the signal to be averaged is offset from ground potential. It achieves this by resetting the input signal to the averaging computer or tape recorder to ground potential whenever a spike occurs. In this way, the signal that is to be averaged can occupy the whole of the input amplitude range of the averager or recorder; that is, the signal is played into the averager at a higher gain, which in turn improves the precision of the resulting average.

In the example given (Figure 3), the SHA enabled the input signal to the averager to be played in at approximately twice the gain that was possible with high-pass filtering. The standard error of the average obtained with the SHA input was then approximately half that obtained with the conventional filtering technique. The standard error gives an

estimate of the reliability of the mean. In this example, therefore, one would need to use 400% more filtered trials than SHA trials to produce a similar level of reliability of estimation of twitch amplitude. This is a clear advantage in situations in which the signal that is to be measured is known to vary with time. For example, if one wishes to measure the change in twitch amplitude of a single motor unit during a fatiguing contraction, the SHA enables successive averages to be made with a minimal number of spikes, that is, with an increased temporal resolution in comparison to the conventional filtering technique.

The SHA circuit also offers a major advantage in ease and accuracy of calibration when it is used for estimation of muscle twitches. The normal method for force calibration is to apply a series of static loads to the strain gauge and to record the steady dc output levels on FM tape or as a series of voltage levels that can be used to calibrate the amplitude of the output from an averager. When the high-pass filtering method is used, one cannot by definition use static loads for calibration signals, while with the SHA circuit, this is a straightforward procedure.

The general shape of the averaged twitch is very similar in both the filtered and SHA records. That is, contrary to our prediction, the filtering procedure did not appear to distort the waveform of the relatively fast-twitch masseter units. We have not attempted to compare the two techniques in averages of slower-twitch units in other muscles, in which the effect of filter-induced distortion is likely to be more prominent.

Finally, two potential traps for users of this device should be mentioned. The first is that the average of the force in the interval preceding the trigger is not reliable, as the input signal in this period is likely to be off-scale for the averager input in many trials. The sudden vertical deflection in the SHA average at the time of the trigger signal (Figure 3) is of course due to the resetting of the input signal to zero volts by the differential amplifier at that instant. Second, it must be remembered that the input signal is reset to zero not only by the trigger spike but by all other spikes as well. Consequently, the use of this circuit must be restricted to situations in which a computer or some other device can be used to exclude trials in which trigger signals occur within the post-trigger interval of interest. When averaging muscle twitches, this is necessary in any event to avoid summation. In our experiments, both the differential amplifier signal and a high-pass filtered ($\tau = 1$ s) force signal are averaged. The former gives the details of the shape and amplitude of the twitch with improved reliability, while the latter gives an acceptable indication of the averaged events before and after the twitch.

This circuit has been used successfully in this laboratory for spike-triggered averaging of muscle force. It would also improve the resolution of experiments in which spike-triggered averaging of nerve cell membrane potentials was used for the determination of synaptic potentials.

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