EFFECTS OF PROLONGED MOTONEURON ACTIVATION ON NEUROMUSCULAR CONTROL

by

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ABSTRACT

To gain further understanding of the neuromuscular control system, the present study focused on the motor unit subsystem and was designed to examine the effect of prolonged motor unit activity on its physiological parameters. Motor unit properties studied included the firing rates of motoneurons, muscle membrane properties, and motor unit twitch profiles. As well, central drive to the motoneuron pool was assessed.

The muscle used in this study was first dorsal interosseous because its function can be isolated by performing abduction of the index finger. In order to assess changes in motor unit characteristics two protocols were followed: (1) subjects maintained a constant rate of firing and (2) subjects maintained a constant force output while motor unit firing was tracked. Surface and intramuscular electrodes monitored muscle and motor unit activities respectively.

Contrary to expectations, neither the constant rate nor the constant force protocols caused a change in the statistical variability of motor unit firing. However, as was expected, power of the electromyogram (EMG) signal and the mean rectified EMG increased, indicating an increase in the central drive to the motor pool. A shift in power from higher to lower frequency of surface EMG spectrum during prolonged activation suggested a decline in the conduction velocity across the muscle membrane. This was also supported by an increase in the width of the motor unit triggered average (MUTA). These experiments have demonstrated that prolonged activation of the unit will indeed alter MUTA's profile, thereby reflecting changes in the membrane properties of the unit. Some twitch profile potentiation was also seen resulting from prolonged activation.

In conclusion, the results of these experiments show no changes in firing rate variability, contrary to results in the literature. There is a clear increase in central drive to the motoneuron pool and slowing of conduction velocity along the muscle membrane. Both potentiation and attenuation of motor unit twitches were observed.

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1/2RT	1/2 relaxation time. Time delay from onset of contraction until a twitch has relaxed to one-half its maximum force (See Figure 2.3).
ACh	Acetylcholine. The neurotransmitter at the neuromuscular junction.
AHP	After-hyperpolarization. An increase in membrane potential following the depolarizing spike phase of an action potential.
AP	Action potential. A signal propagated by neurons and muscle cells. An all-or-none depolarization of the cell membrane.
CNS	Central nervous system. Includes the brain and the spinal cord.
CT	Contraction time. Time delay from onset of contraction to maximum force of twitch contraction (See Figure 2.3).
CV	(1) Coefficient of variation. A measure of the variability of motor unit firing. OR, (2) Conduction velocity. Speed of movement of a muscle action potential across the muscle fibre membrane.
DHPR	Dihydropyridine receptor. A voltage-sensitive receptor on the T tubule membrane. Involved in E-C coupling.
E-C coupling	Excitation-contraction coupling. The complete process starting from (1) the propagation of a muscle action potential across the muscle fibre membrane, to (2) the transmission of excitation from the T tubule membrane to the SR, leading to

	(3) the release of Ca^{2+} and attachment of crossbridges, and finally (4) the movement of crossbridges causing contraction.
EMG	Electromyogram. Record of electrical activity in a muscle.
IR	Innervation ratio. The number of muscle fibres innervated by a motoneuron.
IS	Initial segment. Part of the motoneuron connecting the soma to the axon. This is where the action potential is generated.
ISI	Inter-spike interval. The time between successive spikes of a tonically firing neuron.
MUAP	Motor unit action potential. Net activity of all muscle fibres of a single motor unit.
MUTA	Motor unit triggered average. Unrectified surface EMG is averaged to the firing of one motor unit within the muscle. It represents a MUAP as recorded by surface EMG electrodes.
MVC	Maximal voluntary contraction.
M-wave	Muscle compound action potential. Evoked by single supramaximal shocks to the nerve innervating a muscle, recorded using surface or intramuscular electrodes.
RMS power	Root mean square power. Total power in the entire unrectified EMG signal is expressed as RMS power.
RYR	Ryanodine receptor. A receptor on the SR. Involved in Ca^{2+} release from the SR resulting from E-C coupling.

SD-spike	Soma-dendritic spike. The SD-spike produced in the soma has a long duration after-hyperpolarization and regulates the firing rate of the motoneuron.
SMU	Single motor unit. A motor unit is a single motoneuron and all the muscle fibres it innervates.
SR	Sarcoplasmic reticulum. An organelle of the muscle fibre which stores Ca^{2+}.
STA	Spike triggered average. A method of averaging which uses a natural trigger (e.g., motoneuron spike) to improve signal quality when the signal to noise ratio is poor.
TTL	Transistor-transistor logic. An IC digital logic family where output high is 2.4-5V (typical = 3.5V) and output low is 0-0.4V (typical = 0.2V).
T-tubule	Transverse tubule. An infolding of the muscle membrane (sarcolemma) which mediates transmission of excitation from the muscle membrane to the sarcoplasmic reticulum.

1.0 INTRODUCTION

1.1 The Neuromuscular Control System

All human voluntary movement, from the most basic to the most intricate, uses an incredibly complex neuromuscular control system. This system gives us the ability to move, with such accuracy and agility that when compared, even the most sophisticated of robots appear awkward. Human beings and other animals possess neuromuscular control systems which have evolved to perform the tasks for which they are required, as accurately and efficiently as possible. Feedforward control of muscles during a movement makes the system fast and stable, while feedback corrects for errors and adaptive gain control optimizes sensitivity of the feedback. To illustrate, consider a hiker crossing rough terrain: the placement of each foot is adjusted to compensate for factors such as an increase or decrease in surface grade. In doing so, the neuromuscular system demonstrates its ability to adapt to confounding perturbations. The system is also remarkably durable, since it is able to function reliably throughout the lifetime of most human beings.

These characteristics of human and animal neuromuscular control systems have prompted scientists and engineers to endeavor to understand the systems' complexities and strategies. The practical benefits of doing so are extensive. Medical applications include: development of rehabilitation methods, prosthetic design, neurology, and rehabilitation robotics. Non-medical applications are found in the design of industrial robots and control systems. Here, aspects of characterized biological models may be incorporated into the design (Yamada et al., 1994).

A simple feedforward/feedback model of human voluntary neuromuscular control is diagrammed in Figure 1.1. Voluntary commands from motor areas in the cerebral cortex are carried via descending neuronal pathways to the spinal cord. The signals are transmitted either directly or by way of interneurons to motoneurons which relay the command signal to the target muscles. Sensory feedback within the system may originate from several sources such as force, length, and chemo-receptors, as well as visual, auditory, and vestibular inputs.

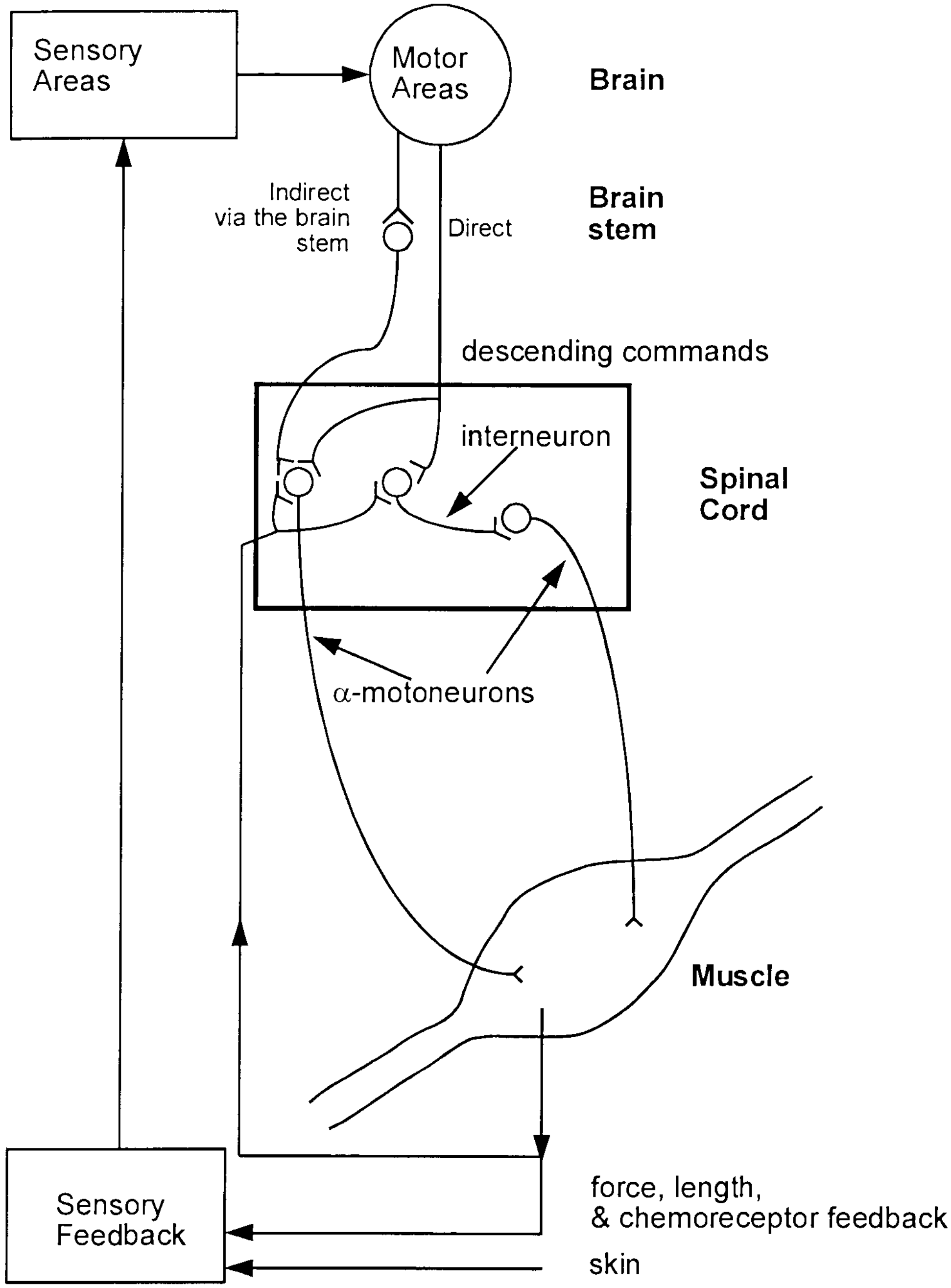

Figure 1.1. Model of human voluntary neuromuscular control.

As just described, there are good reasons for increasing our understanding of neuromuscular control. This thesis project was designed to add to the knowledge base by characterizing a component of the neuromuscular control system under conditions of prolonged activation. That component of the neuromuscular system included the motoneuron and the muscle fibres it innervates. Experiments were designed to isolate this sub-system, thereby allowing evaluation of its response to specific input conditions. In this way, the motoneuron was treated as if it were a black box. In Figure 1.2, the model is shown with three inputs to the motoneuron: (1) the descending command, (2) noise, and (3) feedback. Noise includes interneuronal input (noise of a presynaptic origin) in addition to the inherent properties of the motoneuron (noise of a postsynaptic origin). Inherent properties of the motoneuron, such as changes in ionic conductances of individual channels (thermal noise), cause fluctuations in the resting level of the motoneuron which will affect the probability of the motoneuron firing.

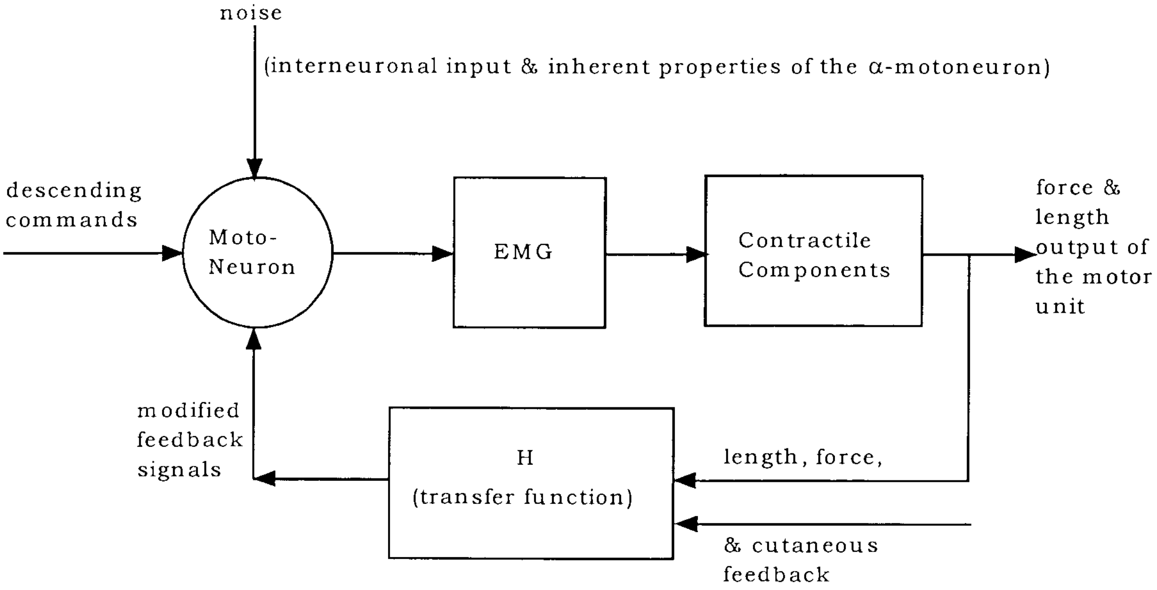

Figure 1.2. Model of a motoneuron element in the neuromuscular control system.

Three components of the system in Figure 1.2, the motoneuron, the muscle membrane (EMG block), and the force generating mechanism (contractile components block), were characterized in this thesis for changes in their properties during prolonged motoneuron firing. The major component of the model and focus of the study is the motoneuron. A motoneuron integrates all inputs and produces a digitized signal for transmission to the muscle. Since integrating properties of motoneurons are reflected in their digital output, any change in analog motoneuron activity due to prolonged firing is reflected in the interspike interval distribution and variability. The block in the system after the motoneuron is labelled EMG (electromyogram). EMG is electrical activity of the muscle membrane resulting from motoneuron input to muscle fibres. An EMG signal depends upon the number of active muscle fibres, conduction velocity of action potentials on muscle fibres, and properties of the recording electrodes. The next block in the model is called the contractile component which is the force generating system. Activity of the contractile components can produce force or length change depending on the loading of the muscle.

Human physiological systems can be examined by the use of linear and non-linear analysis depending on the problem. Linear analysis techniques have been successfully applied to study and model the properties of muscles, motoneurons, spindle afferents, and golgi tendon organs. Since physiological systems are prone to fatigue-induced non-stationarities, one may question the applicability of linear analysis; however, linear analysis remains a valid technique when small time-segments of the data are used, thereby minimizing the non-stationarity. Stationarity is assumed within each segment. The rest of this chapter will deal with the physiological properties of the neuromuscular system and how they are affected by prolonged usage of various components of the neuromuscular system.

1.2 The Motor Unit

A motor unit is defined as a single motoneuron and all the muscle fibres it innervates. All muscle fibres innervated by one motoneuron are called a muscle unit. Motor units differ on the basis on numerous properties, from their size to their biochemical characteristics. Since motor units are the ultimate effectors of the motor control system, motor unit anatomy and physiology must be understood in order to appreciate the system's complexities. The purpose of this section is to fulfill that requirement. This will be done by describing the two components of a motor unit individually, first the motoneuron and then the muscle unit. In addition, section 1.2 includes descriptions of the action potential, motor unit recruitment, and rate coding as they apply to motor unit properties under normal conditions. Section 1.3 details the effect of prolonged activation on the motor unit.

1.2.1 The Alpha Motoneuron

The alpha motoneuron pool is the final path by which electrical control signals reach the muscle fibres in a muscle. Figure 1.3 illustrates a typical spinal motoneuron. Input to the motoneuron is at both the dendritic tree and the cell body (soma). The input may be either excitatory or inhibitory. All inputs are integrated at the soma, and if the total depolarization is great enough, an action potential (AP) is generated at the axon hillock (initial segment). The action potential is an all-or-none signal which rapidly propagates down the axon towards the motoneuron's target muscle fibres. At the same time, the initial-segment (IS) spike travels to the soma and produces the soma-dendritic (SD) spike. The SD spike has an afterhyperpolarization (AHP) of long duration controlling the firing rate of the motoneuron.

Cell bodies of motoneurons lie in the ventral horn of the spinal cord (Truex and Carpenter, 1969). The motoneurons can be excited or inhibited by descending pathways and peripheral afferents, either directly or indirectly via spinal interneurons. In humans, direct connections are frequent between descending pathways originating in the motor cortex and spinal motoneurons. For example, a large number of axons of the corticospinal tract have monosynaptic connections onto upper limb muscles. These connections are stronger in the distal limb musculature and become weaker as one moves proximally (Palmer and Ashby, 1992). Another descending pathway, the reticulospinal tract, has monosynaptic connections betweeen its fibres and the motoneurons activating neck and axial muscles (Rothwell, 1994), but this pathway may not be present in humans. Peripheral afferents, such as spindle Ia and some spindle group II, make monosynaptic feedback connections onto the motoneuron. Descending pathways of the vestibulospinal tract in the cat are disynaptic rather than monosynaptic, which therefore involves the participation of interneurons in the relay of the descending signal. The vestibulospinal tract acts to excite extensor motoneurons and inhibit flexor motoneurons.

Not only is a motoneuron's behaviour or output determined by the nature of its inputs, but also how it integrates the inputs. This integration is determined by the motoneuron's electrical properties such as input resistance and action potential propagation velocity (termed conduction velocity, CV). Size is one of the prime factors affecting motoneuron electrical properties and motoneurons exist in a range of sizes. The relative anatomical size of a motoneuron is determined by a cell's soma and dendrites (Figure 1.3) which, when combined, determine the total membrane area of the cell. Input resistance is dependent upon cell size and specific membrane resistivity. Conduction velocity is dependent upon axonal diameter. Smaller motoneurons have a higher input resistance and a slower conduction velocity than do larger ones. In addition, smaller motoneurons have a higher membrane resistivity (Burke, 1981). Combining size and membrane properties leads to a higher input resistance for smaller motoneurons (Burke, 1981). Therefore, given the same input current, the smaller motoneuron will produce a greater depolarization across its soma membrane. If two motoneurons of a different size receive the same level of input and have the same threshold for action potential production at their axon hillocks, the smaller neuron will begin firing at a lower input level than will the larger neuron. As well, smaller motoneurons have a greater density of inputs than do larger motoneurons which results in the smaller motoneuron receiving a greater input current.

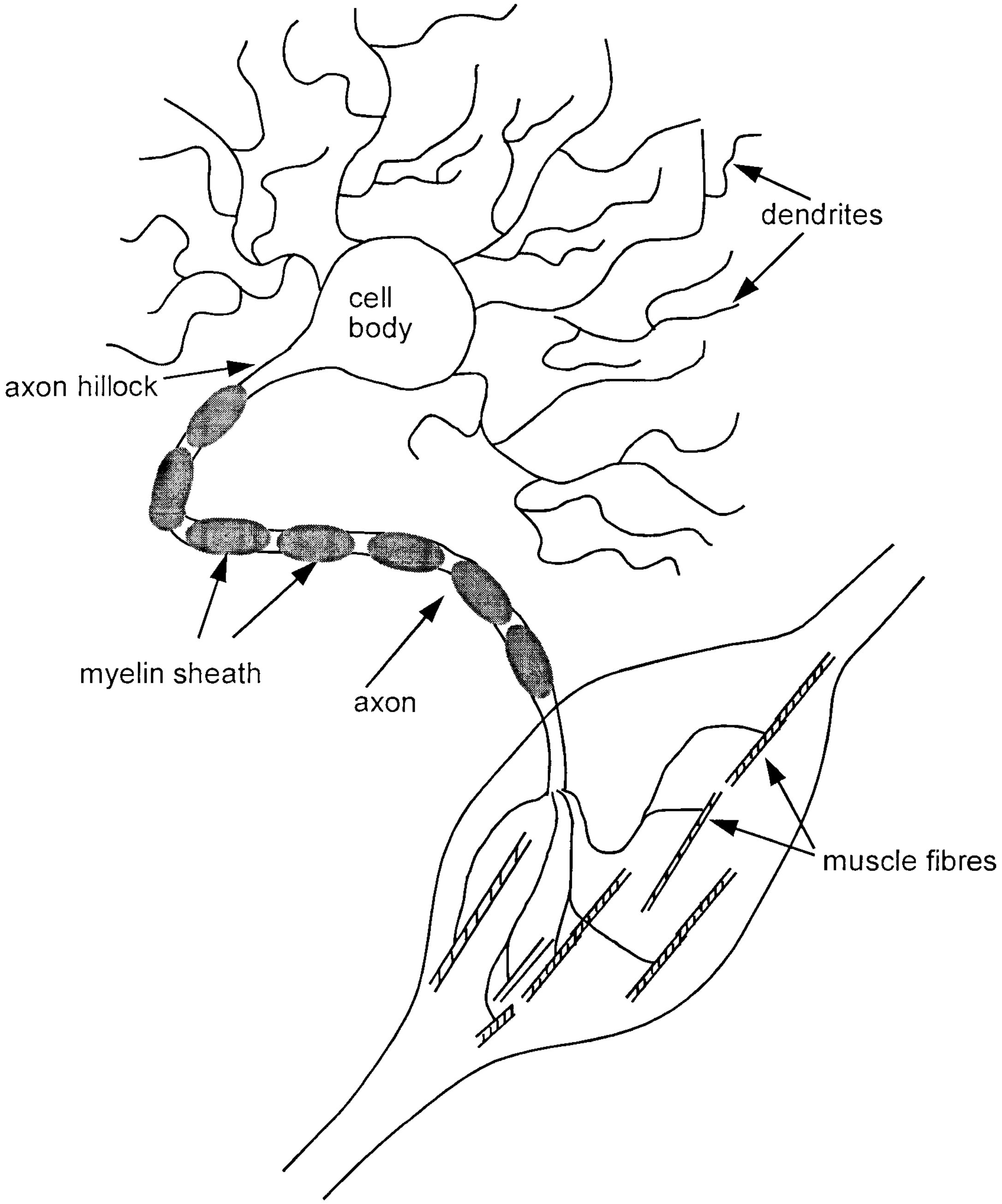

Figure 1.3. Schematic of an alpha motoneuron with the muscle fibres it innervates in the muscle.

In acute experiments (terminal experiments with live animals), a small and a large motoneuron may be differentiated by the size of their axonal action potentials and by the velocity of action potential propagation along the axon. The amplitude of an extracellularly recorded action potential is directly related to the diameter of the motoneuron axon in which it is conducted: the larger the diameter of the axon, the larger its recorded action potential (Clamann and Henneman, 1976). For myelinated axons, conduction velocity is proportional to the diameter of the axon.

A larger motoneuron innervates more muscle fibres than a smaller one (for review see Burke, 1981). The number of muscle fibres innervated by the motoneuron of a motor unit is called its innervation ratio and varies both within a muscle and between muscles, depending upon the muscle size and function (e.g., fine versus gross movements). Muscles for fine control have motor units with low innervation ratios while muscles for gross, powerful movements have high innervation ratios. For example, the extraocular muscles have motor units with innervation ratios of about 10 and the gastrocnemius muscle motoneurons may innervate up to 2000 muscle fibres. At the same time, innervation ratios within a muscle vary: low forces are used for fine control so motor units with low innervation ratios are recruited before those with larger innervation ratios.

1.2.2 Muscle Fibres

As described above, motoneurons exist in a range of sizes and therefore, exhibit a range of biophysical properties. Similarly, muscle fibres have a range of properties. As such, under normal conditions, each motoneuron is matched to the properties of the muscle fibre that it innervates. To illustrate, consider a motoneuron that discharges briefly at high rates and has a low mean daily activity. This type of motoneuron will innervate muscle fibres exhibiting fast contraction times and rapid fatigue. The matching of form and function of motoneurons and muscle fibres is an evolutionary adaptation to usage requirements. Much of this work on differentiating muscle fibre and motor unit types using physiological and histochemical properties was based on animal models such as the cat (Henneman and Olson, 1965; Burke et al., 1973; for review see Burke, 1981).

Prior to the classification of motor units, muscle fibre types had been identified and typed according to histochemical and biochemical characteristics, vascularization around the fibre, and fibre size (Burke et al., 1973; for review see Burke, 1981). The histochemical/biochemical profile indicates the fibre's capacity for anaerobic glycolysis and/or its dependence on aerobic metabolism. The greater the number of capillaries surrounding a muscle fibre, the greater the fibre's dependence on aerobic metabolism. Larger fibres have fewer mitochondria and show less intense ATPase activity than their smaller, darker counterparts (Henneman and Olson, 1965). To this end, the muscle fibre naming system for animals reflects the relative importance of oxidative (O) versus glycolytic (G) metabolism. Table 1 shows one of the well accepted classification systems for muscle fibres and motor units (Burke, 1981). The three fibre types are slow oxidative (SO), fast oxidative-glycolytic (FOG), and fast glycolytic (FG). Prior to this classification system, Henneman and Olson (1965) had found all three fibre types in the cat medial gastrocnemius (MG) but only type SO fibres in the soleus muscle. The classification system for human muscle fibres uses a different naming system to that developed for animals, all the same, the fibre type breakdown corresponds to the animal fibre types as shown in Table 1.

Table 1.1 Muscle fibre and corresponding motor unit types.

Muscle Fibre	**animal**	SO	FOG	FG
	human	I	IIA	IIB
Motor Unit		S	FR	FF

In addition to providing further evidence to "support the hypothesis that the histochemical characteristics of muscle fibres are meaningfully related to the physiological properties of the same units," Burke et al. (1973) presented a classification system for motor units which was closely linked to the muscle fibre classifications. This system was developed using two physiological properties: motor unit twitch profile and fatigue sensitivity. Based on these properties, most of the motor units were classified as belonging to one of three major types: slow (S); fast, fatigue-resistant (FR); and fast, fatiguable (FF) (Table 1). An S type motor unit twitch has a longer contraction and relaxation time and a smaller amplitude than the FR and FF motor units. On the other hand, FF units are more fatiguable than either FR and S type motor units. Although the classification scheme for motor units was developed using animals, it has been applied to some human models (for review see Burke, 1981). In Table 1, the motor unit names are the same for animals and humans, but the muscle fibre types are not: type SO in an animal corresponds to type I in a human, type FOG fibre to type IIA, and type FG fibre to type IIB.

For optimal motor unit output, physiological properties of motoneurons and muscle fibres are matched. In other words, motoneuron properties are determined by the type of motor unit that the motoneuron belongs to. For example, motoneurons corresponding to fast, fatiguable motor units have (a) a lower input resistance, (b) a faster conduction velocity, (c) a shorter and shallower after-hyperpolarization (AHP, see Figure 1.4), and (d) a higher innervation ratio than do motoneurons of slow type motor units. The lower input resistance gives the fast-fatiguable motor units a higher threshold for firing. The shorter AHP allows the FF motor units to fire at faster rates, accommodating the fast contraction times of the muscle units. The properties of motoneurons of fast, fatigue-resistant motor units lie between those of slow and those of fast, fatiguable motor units.

1.2.3 Activation of Muscle Fibres

Muscle fibres are activated in response to an action potential which travels down the motoneuron to the target fibres. The action potential is an electrical signal propagated by both nerve and muscle cells. It is a transient all-or-none depolarization of the cell membrane resulting from the selective movement of ions across the membrane.

At the nerve-muscle synapse, known as the neuro-muscular junction, a neuronal action potential causes the release of the neurotransmitter acetylcholine (ACh) from the terminals of the motoneuron. The ACh diffuses across the synapse to activate nicotinic ACh receptors on the end-plate region of a muscle fibre. The channels open to allow Na^+ and K^+ to travel across the membrane. A net influx of Na^+ results in a depolarization of the post-synaptic membrane from its resting potential of -90 mV. This transient depolarization of the post-synaptic membrane is termed an end-plate potential (EPP). Under normal conditions the EPP can be on the order of 70 mV which is more than sufficient to open voltage gated Na^+ and K^+ channels located around the end-plate region and elicit an action potential along the sarcolemma. The action potential travels across the muscle fibre at a rate slower than if it were an unmyelinated neuron due to the additional capacitive load imposed by the transverse tubules (T tubules).

The initial spike of depolarization of the muscle fibre action potential (Figure 1.4) is due to Na^+ moving into the cell through voltage-gated channels. The sharp repolarization is caused by Cl^- moving into the cell (Cl^- has a high conductance). An after- depolarization follows the sharp repolarization. The final phase of a muscle fibre action potential is the after-depolarization which takes the form of a slow repolarization. After-depolarization is due to action potential currents in the T-tubules. A second difference between the profiles of muscle and neuron action potentials is the lack of an after-hyperpolarization in the muscle action potential. This is due to the lower potassium conductance in muscle cells relative to neurons. As well, Cl^- conductance in muscle cells is 10 times the resting K^+ conductance. A low K^+ conductance helps reduce K^+ accumulation in the T tubules, necessary because build-up of K^+ in the T tubule lumen would have a depolarizing effect. For example, during tetanic excitation of the muscle fibre, an increasing accumulation of K^+ in the T tubule would slow the after-depolarization. Without high Cl^- conductance, repetitive activity would result in K^+ accumulation and an increase in the level of after-depolarization. As a result, at a certain level of depolarization, action potential generation would be self-perpetuating. High Cl^- conductance reduces this risk.

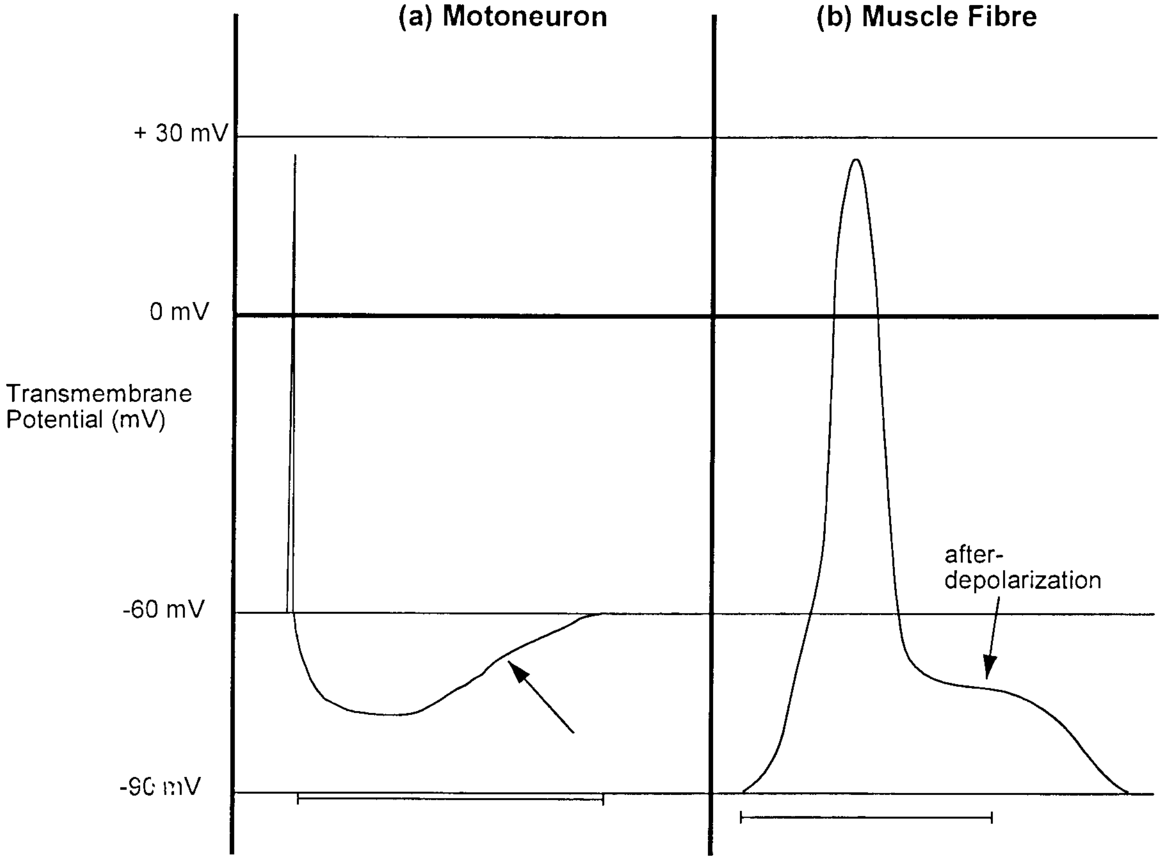

Figure 1.4. Motoneuron and muscle fibre action potentials. Scale is approximately 100 ms for an S type motoneuron and 5 ms for a twitch fibre.

Spread of an action potential across a muscle fibre and into the T tubules will elicit contraction of the fibre's contractile machinery. However, for this to occur, depolarization of the T tubule must be coupled with a release of Ca^{2+} from the fibre's sarcoplasmic reticulum (SR). In vertebrate skeletal muscle, excitation-contraction (E-C) coupling at the triad (a region on the T tubule where the SR comes within close proximity) has been hypothesized to occur via a mechanical process (Block et al., 1988; Bers and Stiffel, 1993). E-C coupling models show a depolarization of the sarcolemma causing a change in configuration of dihydropyridine receptors (DHPR) on the T tubule membrane. The altered configuration of the DHPR molecule signals to the ryanodine receptor (RYR) on the SR to release Ca^{2+}. The magnitude of Ca^{2+} release is proportional to the magnitude of T tubule depolarization.

Ca^{2+} release from the SR induces actin-myosin cross-bridge formation in the muscle's contractile machinery. Muscle fibre contraction occurs with the attachment of myosin heads to sites on the actin molecule. This attachment only takes place in the presence of Ca^{2+}. Attachment of a myosin crossbridge to a particular actin site is short lived and the making and breaking of crossbridges is cyclic. Contraction cycling continues as long as ATP and Ca^{2+} are available. Contraction ceases when Ca^{2+} has been actively pumped back into the sarcoplasmic reticulum.

In slow type muscle fibres, cross-bridge heads on the myosin are not spaced regularly and Ca2+ is pumped back slowly. Fast fibres have a different type of myosin, crossbridges are arranged regularly and calcium is pumped back faster.

To activate a muscle unit, an action potential initiated at the initial segment invades all terminal boutons of the motoneuron at the neuromuscular junction. These action potentials produce action potentials in the corresponding muscle fibres of the muscle unit. The compound action potential resulting from an almost synchronous discharge of all muscle fibres of a motor unit is called the motor unit action potential (MUAP). This should be differentiated from the motor unit spike recorded with intramuscular microelectrodes and from the motor unit triggered average (MUTA, see below) recorded by surface EMG.

1.2.4 Surface and Intramuscular Motor Unit Identification in Humans

To examine the properties of a human motor unit and to study its behaviour under different conditions (for example, fatiguing contractions) requires that the researcher be capable of differentiating the unit from all other active motor units within the muscle. Three signatures are used with intramuscular electrode recordings for motor unit identification: needle recorded motor unit action potentials, motor unit triggered average of the motor unit population's EMG signal (MUTA), and motor unit twitch profile.

In humans, motor unit activity is normally recorded using a pair of fine-wire electrodes inserted into the muscle. A motor unit action potential recorded by a needle electrode is the summated activity of action potentials of the motor unit's muscle fibres. Only those muscle fibres which are sufficiently close to the electrode will have their electrical activity recorded. As a result, the shape of the action potential recorded with a needle electrode is dependent upon the spatial distribution between the muscle fibres and the micro-electrodes as well as the closeness of the electrode to active muscle fibres of a unit. The AP shape will change if the electrodes are moved. When the electrodes are in place, motor units can be differentiated and identified by the shape and magnitude of their potentials. A needle-recorded motor unit action potential is only a sample of the total motor unit action potential (MUAP), the electrical activity of *all* muscle fibres in the motor unit.

A needle-recorded motor unit action potential with distinct size and shape can be isolated and converted into a transistor-transistor logic (TTL) pulse by amplitude-time windows (discriminators). The TTL pulse is then used to extract MUTA and the motor unit twitch profile. MUTA is generated using spike-triggered averaging techniques on the unrectified surface EMG of the muscle (Milner-Brown and Stein, 1975). The motor unit spike is used as a natural trigger. MUTA's shape depends on the spacing and position of the surface electrodes, but once the electrodes are fixed in place, MUTA can be used for motor unit identification throughout an experiment. The relative size or innervation ratio of the motor unit has a strong positive correlation with the amplitude of MUTA (Milner-Brown and Stein, 1975; Palmer and Fetz, 1985; Lemon et al., 1990). Motor unit twitch is the impulse force response of the muscle to a single action potential and is computed by motor unit triggered (using the TTL pulse) averaging of the muscle's force. The twitch profile provides information about the contractile properties of the motor unit (Milner-Brown et al., 1973a).

1.2.5 Recruitment, Rate Coding, and Statistical Properties of Firing

The output of the motor system is enacted through recruitment of motor units and rate coding of motor unit activity. Most of the earlier studies in motor unit recruitment and rate coding used animal models. With advances in technology, intramuscular recordings of motor unit action potentials and the methods of twitch profile and on-line MUTA computation saw the use of human subjects in such studies.

1.2.5.1 Recruitment and The Size Principle

A motoneuron pool is the collection of alpha-motoneurons innervating all of the muscle fibres in a muscle. The size principle states that a pool of motoneurons receives an input and that the strength of the input to the pool determines the number of motoneurons active during a movement (Henneman, 1981). With increasing levels of input to the motoneuron pool, more and more motoneurons are actively recruited in a size-related manner from small to large.

Henneman (1957) first showed that motoneuron excitability is inversely proportional to motoneuron size. In his first of a set of experiments performed on anesthetized cats, he elicited reflexes in lumbar ventral roots by electrical stimulation of the corresponding dorsal root. Stimulation of the dorsal root elicited action potentials which were recorded from ventral root with bipolar electrodes. Henneman observed that as the stimulus intensity increased, more and more motoneurons were recruited and that each new motoneuron had an action potential bigger than all previously activated motoneurons. This showed that motoneuron recruitment proceeds from small to large.

In the next set of experiments, Henneman and co-workers (Henneman et al., 1965) provided much data from the cat to further support the existence of a size principle. The triceps surae muscles of a decerebrate cat were attached by the achilles tendon to a force transducer. As in the preceding experiments, recordings of action potentials were made from ventral root filaments. When the force transducer pulled on the tendon, Ia input to the triceps surae motoneuron pool increased and motoneurons were recruited. As in the experiment with electrical stimulation of the dorsal root, smaller motoneurons were recruited before large, as indicated by the size of their action potentials. Later studies on the decerebrate cat by Bawa and co-workers (1984) showed a correlation between recruitment order and conduction velocity as would be predicted by the size principle.

In 1973 (b), Milner-Brown et al. extended the size principle to humans. Motor unit twitch tension was extracted during voluntary isometric contraction of first dorsal interosseous (1DI). As the subject performed a ramped isometric contraction, motor units were recruited from small to large, which was shown by the magnitude of their associated twitch profile. This study was the first direct evidence of the size principle at work in terms of force output, during human voluntary contractions.

As with all physiological systems, the evolutionary design of the neuromuscular system from which the size principle emerges incorporates numerous advantages over other possible recruitment strategies. First, consider the increase in motor unit force output with increasing drive to the motoneuron pool. Muscles will contract in a smooth manner since recruitment of a new unit will add only a small percentage to the previously existing force. A smooth generation of force allows for greater precision in movement. Secondly, if the muscles or task groups are activated by a common drive, then the need for the motor cortex to control every single motoneuron is eliminated. This arrangement organizes neuromuscular control into a hierarchical system, effectively delegating and focusing levels of control. Henneman and Mendell (1981) showed how the size principle concept simplifies the functioning of neuromuscular control. For example, in a motor unit pool of 300 motoneurons, the total number of possible combinations of motoneuron activation is greater than 10^{90}. Consequently, they postulated that the nervous system would be unable and too slow to deal with this many combinations. Not only does the size principle organize the control of the nervous system into various levels of command, but energy expenditure is also economized. Hatze and Buys (1977) modelled motor unit recruitment with respect to energy expenditure and showed that at all levels of force output, energy expenditure is minimized by motor units recruited according to the size principle.

The universal validity of the size principle has been questioned by numerous researchers, in particular with respect to the existence of task groups[1] (Desmedt and Godaux, 1981; Stuart and Enoka, 1983; Cope and Clark, 1994; Jones et al., 1994). Physiologically identified task groups within muscles are not exceptions to the size principle, since recruitment within the task group is according to motoneuron size (Riek and Bawa, 1992). Spatial arrangement of two task group's muscle fibres may or may not overlap within an anatomically defined muscle (Riek and Bawa, 1992). Others have questioned the validity of exceptions to the size principle (see Cope and Clark, 1994 and Stuart and Enoka, 1983 for a discussion). Some of the exceptions which have been reported involved

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[1] A task group is all of the motor units that contribute to a specific contraction.

nonphysiological conditions (Davies et al., 1993 and Kanda et al., 1977) but such results are questionable with regard to their validity as exceptions to the size principle. Recruitment during lengthening contractions does sometimes seem to bypass small motor units, but the recruitment principle under such conditions is still not clear (Nardone et al., 1989 and Miller et al., 1996). Furthermore, some authors (Stuart and Enoka, 1983) will consider changed recruitment orders caused by positive afferent feedback onto large motoneurons as well as negative afferent feedback onto small motoneurons as exceptions to the size principle. Nonphysiological exceptions to the size principle demonstrate the existence of pathways and mechanisms which can alter the size principle of motor unit recruitment. However, the applicability of these pathways and mechanisms under physiological conditions is largely unproven.

1.2.5.2 Rate Coding

In conjunction with motor unit recruitment, force output of a motor unit, and thus of a muscle or task group, is controlled by varying the rate of motoneuron firing (Person and Kudina, 1972; Milner-Brown, Stein, and Yemm, 1973c; Monster and Chan, 1977; Burke, 1981; Enoka and Fuglevand, 1993; Erim et al., 1996). Control by variation of motor unit firing rate is termed rate coding.

Figure 1.5 illustrates motor unit force versus stimulus rate for both fast and slow type units. A single action potential in a motor unit results in a twitch force. When successive action potentials are applied at a sufficiently fast rate, the twitches fuse to produce a total peak force greater than that of a single twitch. With an increase in the rate of applied action potentials, a level of activation is reached at which further increases in activation rate produce no increase in the force output: this level of force is called tetanic tension. At this point the twitches are fully fused. Different types of motor units have different levels of maximum tetanic tension: a fast-twitch type unit will have a greater maximum tetanic tension than a slow-twitch unit (Rack and Westbury, 1969). Under voluntary contractions we rarely use tetanic firing rates. Even during maximum voluntary contractions, units fire in the range of 20 - 30 impulses/seconds in the upper limbs; motor units do not tetanize at these rates.

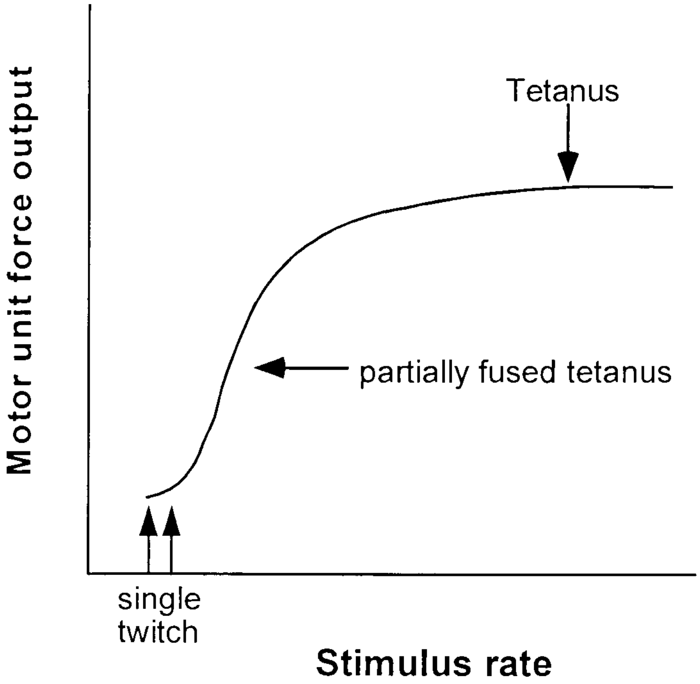

Figure 1.5. Motor unit force versus stimulus rate.

Milner-Brown, Stein, and Yemm (1973c) and Milner-Brown and Stein (1975) studied the relative importance of recruitment versus rate coding on smooth isometric contractions of the 1DI in human subjects. They found that at low levels of force, recruitment of motor units was the major mechanism for an increase in force output (see Figure 1.6). At medium and high force output the dominant mechanism for force increase was through an increase in the firing rate of already active units. In other muscles such as biceps brachii, recruitment and rate coding occur at all levels of force.

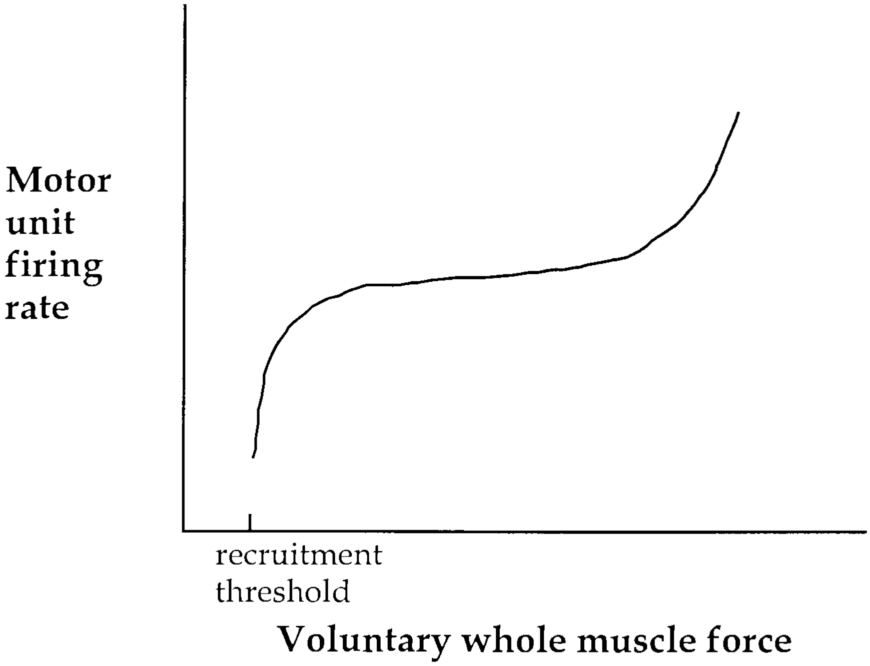

Figure 1.6. Firing rate versus voluntary whole muscle force.

Erim et al. (1996) showed, using the tibialis anterior muscle, that motor unit firing rate will pass through three phases of firing during a linear increase in isometric force output. During the first and third phases, firing rate increases rapidly with a small increase in force. In the middle phase, firing rate increases less rapidly than in the other two phases, but a greater corresponding increase in force is observed. This reduced rate of increase in firing rate may be due to fusion of twitch force. The rapid rate of rate increase with force during the third phase starts at about 70% MVC and may be attributable to the muscle's increasing reliance on rate coding rather than motor unit recruitment to achieve the desired force output. Erim and co-workers also supported previous work (Person and Kudina, 1972; Monster and Chan, 1977) which showed that newly recruited units (those with higher thresholds) fire at lower rates than previously recruited units.

In addition to the level of force output, the size and function of a muscle determine the range of force within which motor unit recruitment dominates over rate coding (Burke, 1981; Person, 1993). For example, small distal muscles tend toward higher maximum firing rates than do proximal muscles. In general, motor units of different muscles exhibit different firing rate ranges. This is expected from Figure 1.5 where slow and fast motor units would fuse at different rates.

Rate coding also plays a role at the start of a contraction when there is a burst of firing which codes for a fast increase in tension. As well, ballistic type movements show higher rates of firing and an increased likelihood of irregular firing (burst firing) than do smooth, tonic contractions (Burke, 1981). The likelihood of burst firing during rapid contractions is greater in fast muscles like the masseter and 1DI than it is in slower contracting muscles such as the soleus.

Short and long-term history of activation affect how motor unit output is rate coded. The burst of 2-4 spikes at the start of a sub-tetanic train of action potentials is an example of short-term history of activation. With the additional impulses tension output is optimized: tension is greater and is reached more rapidly than if the motor unit had fired at a constant rate from the start. This phenomenon, called the "catch-like" property of skeletal muscle, may be due to an increased release of Ca^{2+} from the sarcoplasmic reticulum (Binder-Macleod, 1995). Examples of long-term activation effects on rate coding are posttetanic potentiation and fatigue.

1.2.5.3 Statistical properties of motoneuron firing patterns

Researchers who model motor unit behaviour and neuromuscular control systems are not only interested in mean firing rates[2], but also the firing rate distribution. Knowledge of statistical properties of motor unit firing is necessary for the development of models of neuromuscular control. Clamann (1969) treated motor unit firing as a stochastic (random) point process. In his work based on recordings using biceps brachii, he described motor unit firing as having an "underlying Gaussian probability density." Others (Andreassen and Rosenfalck, 1980; Palmer and Ashby, 1992) reported that the probability density function of motor unit firing was *roughly* Gaussian. That is, Andreassen and Rosenfalck, who used the tibialis anterior muscle of human subjects, described a skewing of the distribution to longer inter-spike intervals (ISI) coincident with a slowing in mean firing rate. Controversy about the shape of ISI distribution arises from the fact that the shape depends upon firing rate: the faster the firing rate, the more closely the distribution appears Gaussian (Jones and Bawa, 1997).

Enoka et al. (1989), described IDI motor unit firing as non-Gaussian. Numerous others simply described the first-order interval ISI histogram as tight and symmetrical at high firing rates but more broad and skewed with a tail to

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[2] Reported mean firing rates for 1DI are 5 - 20 (Kranz and Baumgartner, 1974) and 8 - 20 (Milner-Brown et al., 1973c) impulses per second.

the right (longer ISIs) at lower firing rates (Person and Kudina, 1972; Kranz and Baumgartner, 1974; Gatev et al., 1986; Jones, 1995). Person and Kudina said that the skewed form resembled a Poisson distribution.

Unlike the other experimental protocols, Kranz and Baumgartner required their subjects to maintain a constant rate of firing of the motor unit rather than a constant force for the whole muscle. Subjects were given auditory and visual feedback of the motor unit's action potentials. This method kept the mean rate of motor unit firing stationary. However, even when keeping firing rate constant, there is some inherent variability in motor unit firing. According to Calvin and Stevens (1968), synaptic noise, which changes post-synaptic conductances and produces voltage variations at the axon hillock, is the main contributor to this variability. Less major contributions to variability come from inherent fluctuations in the membrane potential. The effect of noise on motor unit firing properties has been modelled. Matthews (1996) investigated motor unit firing profiles by subjecting a motoneuron model with an exponential after-hyperpolarization trajectory to Gaussian noise. This model provided a realistic representation of human motoneuron firing properties including a decrease in discharge variability with an increase in firing rate.

1.3 Fatigue of the Neuromuscular System

Neuromuscular fatigue has been defined as an impairment in the ability to produce a maximal voluntary force output (Bigland-Ritchie, 1993). This inability to perform a maximum voluntary contraction may or may not affect one's ability to perform tasks at submaximal contraction levels. A fatigued condition has also been defined as an increased perception of the effort required to accomplish the task (Stuart and Enoka, 1992). For example, a subject may be required to maintain a force output of 50% of the maximal voluntary contraction (MVC). At first, the task may seem trivial, until the subject notices that more and more effort is required to maintain the desired 50% MVC. Fatigue has set in. Minutes later the subject will be so fatigued that even a 50% MVC is not possible. This has been defined as the failure point or task exhaustion.

Fatigue is one result of prolonged usage of a muscle. During prolonged neuromuscular activity, motor units fire for extended lengths of time. At some point in time, properties of the motor-system, involved in contraction, begin to change. The objective of the present study was to characterize these changes at the motor unit level.

Previous work on the effects of prolonged motoneuron firing leading to fatigue in force output have used animal models and a constant rate protocol in which the firing rate of motor units is set by a stimulator. Force output is allowed to vary. Human fatigue experiments, on the other hand, have traditionally used a constant force protocol: the subject holds a target force and the firing rate of motor units is free to vary. The intent of this chapter is to review current knowledge on the effect of prolonged usage (especially fatigue-inducing), on the following aspects of neuromuscular control: motor unit firing rate, muscle membrane properties, central drive, and contractile properties.

To review, voluntary skeletal muscle contraction involves the transmission of a signal from the motor cortex to the muscle fibre. The signal is propagated via the spinal cord, to the ventral horn region of the spinal cord where a motoneuron carries the signal to the neuromuscular junction. The signal traverses the junction to the muscle fiber via a chemical neurotransmitter and if depolarization due to the neurotransmitter is sufficient, a muscle action potential is generated which leads to contraction of the muscle fibre. All along the path of transmission are potential sites for signal modification during prolonged usage. Virtually every site, from the brain to the cross-bridge in muscle fibre, has been examined for evidence of fatigue-correlated changes. For example, central mechanisms, such as a reduced central drive to motoneuron pool, are possible causes of the fatigue resulting from prolonged activity. Suspect mechanisms and sites of fatigue also include the motoneuron itself, which is the focus of this study, as it may also show changes in its properties. Some evidence of motoneuron behaviour alterations in response to a constant excitatory input was found in cat motoneurons (Kernell and Monster, 1982b). The neuromuscular junction has also been suggested as a failure point for fatigue. The muscle fibre itself may also be a site of fatigue. In particular, changes in transmission of the signal along the fibre membrane (T tubule transmission), excitation-contraction coupling, calcium release and re-uptake mechanisms from the sarcoplasmic reticulum, cross-bridge formation (including calcium-troponin binding and release), and metabolic factors (e.g., lactate build-up). All will be discussed in greater detail throughout this section. A last suspect mechanism of neuromuscular fatigue is peripheral feedback such as that which occurs in response to metabolite feedback, reducing motoneuron firing rate (Bigland-Ritchie, Dawson, Johansson, and Lippold, 1986). The search for fatigue mechanisms and sites of fatigue is complicated by the probability that both are variable, depending upon the experimental conditions.

1.3.1 Central Mechanisms

Central fatigue is a reduction in the ability to optimally drive the appropriate motoneuron pools to produce the required muscle output. Numerous researchers have searched for evidence that, with prolonged maximal activity, drive to the motoneuron pool will become less than optimal (Merton, 1954; Bigland-Ritchie, Furbush, and Woods, 1986; Bigland-Ritchie, Dawson, Johansson, and Lippold, 1986; Enoka and Stuart, 1992; Gandevia et al., 1995). Conflicting conclusions have been reached, depending upon the muscle group used and the experimental paradigm.

As a signal leaves the motor cortex and travels towards the periphery, it is probably modified and fine-tuned by a variety of association centres in the central nervous system (CNS). The magnitude of this fine-tuned central drive to the motoneuron pool affects not only the firing rates of the motoneurons within the pool, but also the number of active units. Merton (1954) was the first to assess the ability of humans to maintain a maximal drive under fatiguing conditions. He used the stimulus interpolation technique to assess the degree of voluntary activation of the muscle. With this technique, if the drive to the muscle is submaximal, then superimposing maximal electrical stimuli (stimulus interpolation) during an MVC will generate extra force. Merton used the adductor pollicis muscle on human subjects. Since, during fatigue, a maximal stimulus produces the same tension as a maximal voluntary contraction, he saw no evidence of submaximal central drive and thus concluded that fatigue is peripherally induced.

Two studies published in 1986 by Brenda Bigland-Ritchie and her colleagues supported Merton's 1954 findings that fatigue is not a result of inadequate central drive (Bigland-Ritchie, Dawson, Johansson, and Lippold, 1986 and Bigland-Ritchie, Furbush, and Woods, 1986). Bigland-Ritchie, Furbush, and Woods used methods similar to Merton's (1954) for submaximal voluntary contractions (50% MVC). In this study subjects performed intermittent submaximal isometric voluntary contractions of the quadriceps, adductor pollicis, and soleus muscles. Central drive was evaluated by superimposing single maximal shocks on target force voluntary contractions, relaxed muscle, and maximal voluntary contractions as well as evaluation of the integrated surface EMG of the muscle. Some evidence was seen of muscle-specific fatigue mechanisms: experiments on quadriceps and adductor pollicis showed no evidence of the central drive being less than maximal during the fatiguing tasks, but those on soleus did. Throughout the experiments on the quadriceps and adductor pollicis muscles, superimposed twitches could not be detected when shocks were applied to the MVCs (this implies that the muscle was maximally activated). During the same experiments, when the twitches were superimposed on the submaximal target force the twitch magnitudes declined rapidly and were undetectable at the limit of endurance when the maximal voluntary contraction became the target force. The results were not so clear for soleus. Experimental measurements showed that when the limit of endurance was reached (when the target force because the MVC due to fatiguing processes) the muscle was only approximately 77% activated by maximum voluntary effort. Differences in fatigue resistance between the three muscles likely caused the dissimilar results. However, in all three muscles the primary cause of a decrease in force output was attributed to contractile apparatus failure.

In the second study, Bigland-Ritchie, Dawson, Johansson, and Lippold (1986) showed that during maximal voluntary contractions, the firing rates of motor units decrease. More precisely, they used maximum voluntary elbow flexion to test whether a reduction in discharge frequency during a fatiguing process is due to "intrisic processes in the CNS" or due to peripheral reflexes. Their protocol involved comparing recovery of firing rates under ischaemic conditions versus under intact blood flow conditions. Following a fatiguing contraction, motor unit recovery was monitored for three minutes, long enough for any central changes to return to normal. The researchers found that recovery was complete when the blood flow was intact whereas ischaemic conditions inhibited recovery. Because recovery was inhibited peripherally (by restriction of blood flow to the muscle), these experiments suggest that during fatiguing conditions, motoneuron firing rates are affected more by peripheral reflexes than by decreasing central drive.

Enoka and Stuart (1992) found evidence of exceptions to the idea that humans can produce an adequate central drive during fatiguing activity. First, unless a subject is well practiced and motivated, the neural drive will probably be submaximal during the activity (Bigland-Ritchie, Kukulka, Lippold, and Woods, 1982; Bigland-Ritchie, Johansson, Lippold, and Woods, 1983). Second, some non-fatigued muscles are less easily maximally activated than others (e.g., tibialis anterior is easier to activate maximally than are ankle extensor muscles) due to varying strengths of connection between the CNS and the motor units innervating different muscles. Last, the type of task affects the ability to recruit maximally. For example, the level of activation depends on the speed of contraction. In addition, subjects have more difficulty activating all motor units for a concentric[3] versus an eccentric[4] MVC.

Bellemare and Bigland-Ritchie (1987) also produced evidence of inadequate central drive during fatiguing contractions. Central drive was evaluated using the ratio of the superimposed twitch on the target force to the superimposed twitch on the relaxed muscle. The diaphragm muscle was used and twitches were applied to the phrenic nerve. The results showed that once a target submaximal force could not be reached as a result of fatigue, one half of the reduction in the MVC could be attributed to reduced central motor drive while the rest was due to contractile apparatus failure.

Cortical magnetic stimulation has been used in conjunction with the experimental design of Bigland-Ritchie (Bigland-Ritchie, Dawson, Johansson, and Lippold, 1986) to determine at which location a reduced central drive may

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[3] In a concentric contraction, the muscle shortens as it actively contracts.

[4] In an eccentric or lengthening contraction, the muscle lengthens as it actively contracts.

be operating (Gandevia et al., 1995). Maximal voluntary drive or effort was evaluated using twitch-interpolation techniques with both cortical magnetic and motor point stimulation. The results showed that following maximal voluntary contractions, motoneurons and the motor cortex return immediately to their non-fatigued state. This result prompted Gandevia and co-workers to suggest that reduced output in centres "upstream of the motor cortex" cause an insufficient neural drive and therefore contribute to the development of central fatigue. As well, Gandevia et al. (1995) have shown that the peripheral reflex, proposed by Bigland-Ritchie, is long rather than short-looped because it provides feedback to centers in the brain, feeding to the motor cortex. This reflex probably involves joint, muscle, and tendon afferents (see Bigland-Ritchie, Dawson, Johansson, and Lippold, 1986 for a discussion of the peripheral reflex). Gandevia's group also concluded that changes in alpha-motoneuron excitability contribute little to a decrease in force output during fatigue.

Also of relevance to the role of central drive in fatigue processes are the notions of sense of effort and sense of force. Sense of effort is the perceived effort or voluntary drive to the muscle whereas sense of force is the perception of actual force output. Jones (1983) found that both sense of effort and sense of force during fatiguing processes are a result of corollary discharge. In his study looking at the effect of fatigue on perception of force, he found that fatigue causes a central mediation of force information. Subjects were asked to hold submaximal contractions of the biceps of one arm until fatigued. Meanwhile, as the arm was becoming increasingly fatigued, the subjects were required to match the force output of the fatiguing arm with their non-fatiguing arm at regular intervals. As the arm became more fatigued, the subjects overestimated the force output by an increasing amount. This growing error in matching forces was suggested to result from increasing central drive with increasing fatigue. Sense of effort is due to a corollary discharge of the output from the motor to the perception area of the cortex. When the central drive increases, the corollary discharge increases, there is then an increased sense of effort, which can be interpreted as an increased feeling of fatigue. Due to this increase in corollary discharge, perceived force output during fatigue is centrally rather than peripherally mediated.

1.3.2 Fatigue and Motoneuron Firing Rate and Rate Variability

Under certain experimental conditions, prolonged activation results in a decrease in motoneuron firing rate. This decrease in rate is viewed as an intentional down-modulation of firing rate by the nervous system, serving to optimize control and output of fatiguing muscles. Numerous experimental models, both animal and human, have addressed this question; the results of which are often conflicting.

Some animal studies suggest that inherent motoneuron properties affecting firing rate may contribute to changes in motoneuron firing rates during prolonged activation and are therefore linked to fatigue processes. For example, "the pattern of spike-frequency adaptation may be correlated with a number of other motor unit properties including fatigue" (Sawczuk et al., 1995). Kernell and Monster (1982 a&b) used cat gastrocnemius motoneurons to study adaptation of motoneuron firing rates. In addition to an initial drop in firing rate after the start of firing, the rate decreased during the first 30-60 seconds of firing. This decrease in firing rate was called late adaptation. Late adaptation is caused by the "cumulative after-effects of the discharging spikes" (Kernell and Monster, 1982 a&b). Conceivably, the slowing of motoneuron firing rates through late adaptation could contribute to the optimization of force production in the presence of fatiguing conditions (Sawczuk et al., 1995). For instance, fatigue-induced changes in the contractile machinery may not require that a motor unit fire maintain the same fast firing rate for optimum force production of its contractile machinery. So, although late adaptation is an inherent property of the motoneuron alone, it does impact motor unit behaviour during fatiguing conditions.

The decrease in firing rate is reflected by increases in mean ISI and therefore, a shift of the first-order interval histogram to the right (Bigland-Ritchie, Johansson, Lippold, Smith, and Woods, 1983; Gatev et al., 1986; and Enoka, Robinson, and Kassev, 1989). Pre- and post-fatigue first-order interval histograms are described as non-Gaussian and skewed to the right for both submaximal and maximal voluntary contractions. Bigland-Ritchie et al. suggested that a decrease in mean firing rate during maximum voluntary contraction (MVC) conditions implied a left shift in the force-rate curves (Figure 1.5) for muscle (see also Moxham et al., 1982). A left shift is intuitive given the slowing of contractile properties of muscle fibres with fatigue. This phenomenon is called muscle wisdom. Under fatigued conditions, a lower rate of stimulation is required to achieve tetanus, therefore, a left shift in the force-frequency curve would optimize force production. However, conflicting results were reported by Thomas (1995) who saw a right shift in the force-frequency curve in experiments using human triceps brachii. Under quite a different experimental paradigm, Miller et al. (1996) failed to observe a trend in the direction of change in triceps brachii motor unit firing rate during fatigue (as fatigue developed, the mean number of motor unit spikes increased, decreased, or stayed the same).

1.3.3 Neuromuscular Junction Transmission Failure

A number of authors have rejected the hypothesis of neuromuscular transmission failure as a fatigue inducing mechanism. Merton (1954) examined the possibility of transmission failure contributing to fatigue, but this idea was not supported by his experiments. He applied low through high electrical stimulation (until the stimulation was too painful) to the ulnar nerve during voluntarily induced muscle fatigue and recorded the M-wave, an EMG record. As maximal voluntary force declined due to fatigue, the electrical shocks were superimposed on the voluntary contraction in an attempt to restore any of the lost force. Since the M-wave did not change in amplitude with the superposition of the shocks on the voluntary effort, evidence of electrical failure was not seen and Merton concluded that transmission of the signal was not affected by fatigue. Similar results were obtained by Bigland-Ritchie and co-workers (1982, 1983) in studies which used adductor pollicis and 1DI muscles. This work also showed that central mechanisms are unlikely causes of fatigue.

Contrary conclusions were reached by Fuglevand et al. (1993) in submaximal force studies using 1DI. The muscle was fatigued by abducting it at one of three levels: 20, 35, and 65% of MVC. Pulses of a supramaximal level were applied to the ulnar nerve before and following the fatiguing task. A decrease in the M-wave amplitude due to fatigue prompted the conclusion that neuromuscular propagation was impaired. This impairment was greatest for the lower forces.

1.3.4 The Effect of Fatigue on Muscle Membrane Properties

(i) Analysis Using Mean Rectified Surface EMG and Power Spectra

Surface EMG is the interference pattern of the electrical activity of muscle fibres. The recorded interference pattern results from asynchronous firing of many motor units. Under non-fatigued conditions, integrated EMG and force are related monotonically. EMG has long been investigated as a source of parameters to measure the extent of fatigue. In particular, during fatigue there are associated changes in EMG magnitude and frequency composition. During sustained submaximal contractions, EMG amplitude and mean rectified EMG increase (Moritani, Nagata, and Muro, 1982; De Luca, 1984; Fuglevand, Winter, Patla, and Stashuk, 1989; Krogh-Lund and Jørgensen, 1992; Fuglevand, Zackowski, Huey, and Enoka, 1993). The degree or rate of EMG increase depends on the muscle and its relative composition of slow versus fast twitch (group II) muscle fibres. For example, biceps brachii, which has a greater percentage of group II fibres than soleus, has a faster rate of rise of integrated EMG than does soleus (Moritani, Nagata, and Muro, 1982). However, the reliability of EMG amplitude for use as an index of fatigue has been questioned by some researchers (Petrofsky et al., 1982; De Luca, 1984). For example, with fatigue, MVCs result in a decrease in EMG amplitude (Komi and Tesch, 1979) while the effect of a submaximal contraction on EMG amplitude will depend on factors such as the muscle, the %MVC of the contraction, and whether recruitment of all motor units has taken place.

Another measure of fatigue, for both submaximal and maximal conditions, is a downward shift in mean and median frequency of the EMG power spectrum (Komi and Tesch, 1979; Moritani, Nagata, and Muro, 1982; Petrofsky, Glaser, Phillips, Lind, and Williams, 1982; De Luca, 1984; Fuglevand, Winter, Patla, and Stashuk, 1989; Krogh-Lund and Jørgensen, 1992; Fuglevand, Zackowski, Huey, and Enoka, 1993). The mean power frequency is the frequency at which there is the average amount of power while the median power frequency is the frequency at which the spectrum is divided into two regions with equal power. Mode frequency was also considered for use as an indicator of fatigue but it is not as useful as either mean or median frequency because of the stochastic nature of the EMG signal. Because the power spectrum does not have a sharply defined peak, the mode frequency cannot be determined reliably (for review see De Luca, 1984).

Possible explanations for the increase in EMG amplitude and the frequency shift in the EMG power spectrum during submaximal fatiguing contractions should be considered. Since the EMG signal is a temporal and spatial summation of motor unit activity in the muscle, the following are possible reasons for changes in EMG: recruitment of additional motor units, motor unit synchronization, a decrease in muscle fibre conduction velocity (CV), and the variability of motor unit firing rate. All factors excluding muscle fibre conduction velocity would affect the power spectrum between frequencies of 0 to 40 Hz while frequencies above 40 Hz are affected by changes in conduction velocity (typically in the range of 100 to 200 Hz). Although shifts in mean power frequency are seen, recruitment is not a significant factor in EMG frequency shift during high forces of contraction due to reduced recruitment at such levels (Milner-Brown, et al., 1973). Furthermore, mathematical modelling indicates that synchronization of motor unit firing is not a significant contributor to EMG frequency shift (Stulen and De Luca, 1981).

Bigland-Ritchie, Donovan, and Roussos (1981) investigated the suggestion of a connection between a slowing of the muscle fibre conduction velocity (CV) and a downward shift in surface EMG power spectrum. Using a cooled adductor pollicis to simulate the fatigued condition, they showed that, although a decrease in conduction velocity shifts the power spectrum towards the lower frequencies during an MVC, the decrease in CV alone cannot completely account for the spectral shift. EMG signal power was compared between a high (130 - 238 Hz) and a low (20 - 40 Hz) passband. A decrease in conduction velocity as well as a spectral shift to the lower frequencies was observed. However, when the cooled muscle (cooled to decrease the CV of an unfatigued muscle) was compared to the fatigued muscle, they found that the change in CV required to produce equal changes in EMG was about ten times greater in the unfatigued muscle than the fatigued muscle. A decrease in conduction velocity lengthens the muscle action potential. Since 1981 a number of authors have demonstrated a connection between EMG frequency shift and a decrease in muscle fibre conduction velocity, contradicting the 1981 findings of Bigland-Ritchie and colleagues (Moritani, Nagata, and Muro, 1982; De Luca, 1984; Krogh-Lund and Jørgensen, 1992). Krogh-Lund and Jørgensen estimated that a 12% drop in conduction velocity in brachioradialis caused one-third of the 33% drop in median power frequency of the EMG power spectrum. They could not do the same for biceps brachii and attributed much of its frequency shift to increased synchronization.

The low frequency end of the spectrum (0 - 40 Hz) is also affected by a couple of significant factors. First, more EMG signal is passed at the lower frequencies because body tissue acts as a low pass filter. Second, the lower frequencies are affected by the variability of motor unit mean firing rate (for discussion see De Luca, 1984). Englehart and Parker (1994) used modelling to demonstrate how non-stationarities in the action potential train of a single motor unit affect low frequency regions (0 - 40 Hz) of the single motor unit myoelectric power spectrum. Another method of evaluation of fatigue using EMG is the high to low ratio (e.g., high frequency band of 130 - 238 Hz and low frequency band of 20 - 40 Hz). This is a ratio of EMG power in a high frequency band to one in a low frequency band. Since fatigue causes inconsistent changes in both the high and low frequency bands (e.g., due to recruitment of motor units with significantly different action potential shapes), the ratio is not a reliable index of fatigue (Moxham et al., 1982).

(ii) Motor Unit Triggered Average of the Surface EMG

Membrane events associated with fatigue would be expected to alter motor unit action potentials (MUAPs) and therefore the shape of MUTA. A muscle action potential's profile is affected by potassium accumulation in the T tubules which depolarizes the tubular membrane and impairs action potential conduction into the tubule (Edman, 1995). At the same time, motoneuron action potentials arriving at the neuromuscular junction have become broader with fatigue (for review see Burke, 1981).

The aforementioned effects of fatigue on motoneuron action potentials and MUAPs are reflected in muscle mass action potentials (M-waves) which are evoked by single supramaximal shocks to the innervating nerve. With fatigue, the duration and total area of the M-wave increases (Bigland-Ritchie, Kukulka, Lippold, and Woods, 1982) and one should therefore expect to see a corresponding broadening in MUTA. The broadening of the M-wave is attributable to a slowing of the muscle fibre conduction velocity or due to desynchronization of motor units.

1.3.5 Contractile Properties of the Motor Unit

Motor unit twitches, extracted using a method of spike-triggered averaging (STA), are used to evaluate a unit's contractile properties in humans (Milner-Brown et al., 1973a). Three parameters are obtained from a twitch profile: (1) twitch amplitude, (2) contraction time, and (3) relaxation time (or 1/2 relaxation time). Over time, prolonged muscle activity will change twitch parameters; however, these changes are inconsistent and depend upon the history of motor unit activation.

Typically, a fatigued motor unit is characterized as one whose twitch decreases in both amplitude (force) and increases in duration while the action potential train of the motor unit remains essentially unchanged over a long period of activation. Twitches do not consistently decrease in amplitude following prolonged activity. Sometimes, at submaximal levels of activation, twitch amplitude will increase after a period of time. This is called postactivation potentiation and is similar to posttetanic potentiation (Burke, 1973; Burke, 1981).

Experimental results to date have shown unpredictable twitch responses to prolonged firing (Thomas, 1995). Whether an increase or decrease in twitch amplitude is seen, as well as how long twitch potentiation is sustained, is variable. In a study using the masseter muscle, Nordstrom and Miles (1990) failed to show a correlation between (a) initial twitch force or (b) contraction time and twitch force loss with fatiguing activity. Investigators must be cautious when using spike-triggered averaging because if the time period selected is too long, the averaging process could potentially mask any twitch fatigue or potentiation because twitch potentiation or attenuation could be transitory.

The potentiation or attenuation of a twitch profile following prolonged motor unit activation has been attributed to a number of factors. Calcium kinetics could play a role. For example, the time course of calcium release from the sarcoplasmic reticulum as well as binding and dissociation from regulator proteins may affect excitation-contraction coupling, and hence the contractile properties of the unit (Burke, 1981; Viitasalo and Komi, 1981; Enoka and Stuart, 1992; Edman, 1995). The relaxation time should reflect the rate of re-uptake of calcium by the sarcoplasmic reticulum (Viitasalo and Komi, 1981). Burke (1981), on the other hand, suggested that a slow removal or re-uptake of calcium may be what causes posttetanic potentiation. However, at moderate levels of fatigue (capable of approximately 70% of rested state force), calcium may not be a factor. Edman (1995) shows that a decline in performance at such levels may be due to changed kinetics of the myosin cross-bridges. He does suggest that at excessive levels of fatigue, calcium kinetics may play a role in failure of activation of the contractile system. Using caffeine, Edman demonstrates how, during fatigue, a twitch may potentiate while total muscle force declines. Caffeine will potentiate an unfatigued twitch, but it will not potentiate a tetanus. An application of caffeine promotes the release of calcium from the sarcoplasmic reticulum, indicating an E-C coupling problem. In the fatigued state, surplus calcium is released which produces a potentiated twitch. Yet the tetanus, and hence the total muscle force, does not exhibit a similar increase in amplitude because the contractile system is maximally activated during tetanus.

Potassium ion accumulation in the T tubules is another possible factor in the attenuation of motor unit twitches (Stuart and Enoka, 1992; Edman, 1995). Excitation-contraction coupling failure has also been linked to an increased concentration of hydrogen ions (metabolic by-products) and a reduction in phosphocreatine supplies. The impact of these factors is probably dependent on the fibre type.

1.3.6 Metabolic Factors

Both a diminished energy supply and an accumulation of metabolites have been linked to fatigue.

The first factor, an insufficient energy supply was rejected by a number of researchers (Grisdale, Jacobs, and Cafarelli, 1990; Sahlin, 1992). Grisdale and co-workers addressed this question by examining the effect of energy (glycogen) stores on fatigue during submaximal exercise. Their evidence suggested that previous exercise has a greater impact on muscle fatigue and endurance capacity than do glycogen stores. They proposed that fatigue due to previous exercise is a result of central attenuation of the neural drive, peripheral feedback onto the central drive, or other unknown peripheral consequences of activity.

The currency of energy within the cell, ATP, has also been linked to fatigue. As Sahlin wrote in his 1992 review, "a classical theory of fatigue is that at a certain point of the exercise ATP consumption cannot be met by an equal rate of ATP formation, resulting in an energetic deficiency and impaired force generation." However, since the relative amount of ATP depleted during fatiguing exercise is small ($\leq 40\%$), this hypothesis was rejected (Sahlin, 1992). There is a connection between increased ADP concentration and fatigue. The decrease in pH during fatigue (due to the accumulation of H^+, another metabolic product) slows the rate of ADP rephosphorylation, causing a build-up of ADP (Sahlin, 1986). Although the amount of ATP depleted is small, the corresponding relative increase in ADP and inorganic phosphate (P_i) is large. A slow rate of ADP rephosphorylation may block activation or excitation-contraction coupling (Sahlin, 1986).

Besides slowing rephosphorylation of ADP, a decrease in pH is a suspect fatigue mechanism in a few more ways. Hermansen (1981) suggested that during maximal exercise, when the pH decreases from pH 7.0 to around pH 6.5, the contractile mechanisms of the muscle fibres are affected. The increased acidity (acidosis) inhibits ATPase enzyme activity (and virtually every other metabolic enzyme) which will therefore adversely affect cross-bridge formation for force generation. In addition, the changed environment may alter the concentration of sarcoplasmic calcium or change the response of contractile proteins to calcium. Hermansen (1981) proposes a dual effect of hydrogen ion concentration as both a regulator of ATP utilization and resynthesis. By regulating ATP utilization, the contractile or force-generating apparatus is affected. Sahlin (1992), on the other hand, suggests that a decrease in pH is unlikely to directly affect the contractile mechanisms of the muscle fibre but, rather the rate of glycolysis could be slowed which would affect the energy supply. In addition to H^+, P_i, and ADP accumulation, metabolic by-products H_2PO_4 and lactate are also linked to fatigue (Howell-Fulton, 1989). Grisdale et al. (1990) rejected outright the proposition that fatigue is caused by a build-up of metabolic products such as lactate and hydrogen ions. Enoka and Stuart (1992) propose another possible fatigue mechanism: the depletion of sodium ions and the accumulation of potassium ions in the extracellular spaces surrounding the muscle fiber which would reduce the excitability of the muscle fiber membrane.

1.3.7 Peripheral Feedback

Merton (1954) originally proposed the idea that motoneuron firing rates are, to a certain degree, controlled by a peripheral reflex. In 1986, Bigland-Ritchie and colleagues proposed that alpha-motoneuron firing rates are decreased by inhibitory afferent feedback from group III and IV nerve endings. Later work by Bigland-Ritchie et al. (1992) explored how motoneuron firing rates adjust to suit the fatigue-induced slowing of muscle unit contractile properties in humans. Using muscle cooling to simulate fatigued muscle fibres (cooling slows the rates of contraction and relaxation in the same way as fatigue), they found that cooling did not cause the same decrease in motoneuron firing rate as did fatigue. Bigland-Ritchie concluded that mechanical proprioceptor output from group Ia afferents (Ia firing was not tested directly) does not cause the slowing in motoneuron firing rates seen during fatigue and that perhaps this slowing is attributable to group III or IV free nerve endings which are sensitive to metabolites.

1.3.8 Task Dependency of Fatigue Mechanisms

As illustrated by the experiments described in the section on central fatigue, controversy exists as to whether or not (or to what degree) a decrease in central drive is a cause of fatigue. This discrepancy of conclusions may be attributable to factors such as the type of activity and the muscle(s) used. Indeed, the type of muscle contraction performed, be it maximal or submaximal, determines the mode of control exerted by the central nervous system (Gandevia, 1992). The CNS is faced by two different control problems when a submaximal activity (versus an activity at maximal effort) is performed for long periods. For example, with maximal exercise, whole muscle relaxation and contraction times (contractile properties) have been shown to slow whereas, submaximal exercises may produce an increase in relaxation and contraction speeds (Gandevia, 1992; Bigland-Ritchie, 1993). Due to changes in muscle contractile properties, the temporal characteristics of motor units have been observed to adapt to suit fatigued contractile characteristics. Therefore, not only does the type of contraction determine specific motor unit temporal characteristics in the pre-fatigued condition, but it also determines the fatigue response of motor units. Modification of the descending drive to the motor units is a part of this process (Enoka and Stuart, 1992; Gandevia, 1992).

1.4 Objectives

The main objective of the present study was to examine concomitant changes in multiple subsystems of the neuromuscular control system during prolonged usage. The four components of the system which were the focus of this study were (1) the motoneuron, (2) central drive, (3) muscle unit electrical properties, and (4) muscle unit mechanical properties (Figure 1.2).

The first unit or subsystem to be studied was the motoneuron. Since there is contradictory information (see section 1.3.2) on the changes in firing characteristics of motoneurons during prolonged firing, the objective of this study was to examine (1) the changes, if any, in the variability of the interspike interval when firing rate is held constant, and (2) changes in firing rate when force is held constant.

It is well known that during prolonged firing, the central drive to motor units increases. The second component of the neuromuscular control system under study was central drive. These experiments sought to compare, using surface EMG, the changes in central drive during (a) constant firing rate and (b) constant force paradigms. In human studies, many researchers have addressed the question of how central drive changes with fatigue by using constant force protocols. Animal studies, on the other hand, used a constant stimulation protocol because motoneurons could be directly stimulated. This experiment bridges the gap between those animal and human experiments by using a constant rate protocol on humans.

The third unit under study was the muscle unit. During prolonged firing one expects that there may be changes in the membrane properties of the muscle fibres. These may be reflected in the power spectrum of EMG, MUTA and maximum M-wave. In the literature, the power spectrum mean and median frequencies shift to the left with increasing fatigue (Komi and Tesch, 1979; Moritani, Nagata, and Muro, 1982; Petrofsky, Glaser, Phillips, Lind, and Williams, 1982; De Luca, 1984; Fuglevand, Winter, Patla, and Stashuk, 1989; Krogh-Lund and Jørgensen, 1992; Fuglevand, Zackowski, Huey, and Enoka, 1993). Changes in MUTA with prolonged firing have not been documented however, M-wave, a related measure, broadens with fatigue due to slower conduction velocity of the muscle fibre action potential across the sarcolemma (Bigland-Ritchie, Kukulka, Lippold, and Woods, 1982). The objective was to examine how EMG power spectral shift compares for constant rate versus constant force protocols. As well, MUTA was evaluated for changes due to prolonged firing and/or fatigue. M-waves were also examined for fatigue effects relating to changes observed in MUTA.

The fourth unit of the neuromuscular control system considered in this study was the force generating system. In general, fatigue is defined as an inability to produce force, yet at the same time, it is also well documented that motor unit twitches potentiate during prolonged firing (Burke, 1981; Bigland-Ritchie, Furbush, and Woods, 1986; Thomas, 1995). The objective of the present study was to examine changes in twitch profile as well as potentiation or attenuation during prolonged firing. Was potentiation due to the contractile machinery or due to synchronization of motoneuron firing?

Next, Chapter 2 presents the methodology used to address the above questions.

2.0 METHODS

2.1 Subjects

Experiments were carried out on the right hand first dorsal interosseous (1DI) muscle (refer to Appendix A for a description of the muscle) of five male and three female right-handed volunteers, ranging in age from 24 to 52 years. All experiments received prior approval from the Ethics Committee for Human Experiments at Simon Fraser University. All subjects were free of neuromuscular disorders.

2.2 Equipment

The following four variables were monitored and recorded during the experiments: (1) single motor unit (SMU) electrical activity, (2) Surface EMG, (3) absolute force (DC force), and (4) fluctuations in force (AC force). Figure 2.1 illustrates the positioning of the electrodes on the hand and the recording system, the details of which are described in subsequent sections.

2.2.1 Single Motor Unit Recording

Electrical activity of single motor units was recorded using an intramuscular bipolar needle electrode with a ground electrode attached to the upper arm. All electrodes were fabricated in the laboratory using two lengths of 30-50 μm Teflon coated stainless steel wire (California Fine Wire Company) inserted into the barrel of a 25 gauge needle. The wires were held in place with a biocompatible epoxy. Electrodes with wires lying approximately two to three wire diameters apart in the needle tip were typical of those used throughout the experiments.

The two wires in the needle electrode were connected to a preamplifier set with a gain of 100 (either a NL 824 Neurolog System with a low-end cutoff frequency of 30 Hz or a Grass preamplifier with a passband of 30 - 100 kHz). Output of the preamplifier was then connected to a Neurolog System NL 820 isolator (Digitimer Ltd.) which protected the subject from accidental shock. Isolator output was 100 Hz high-pass filtered by a Wavetek Rockland Model 452 Dual Hi/Lo Filter. The signal was then amplified on the order of 50 times by a conditioning amplifier. A ground electrode was wrapped around the right biceps of the subject.

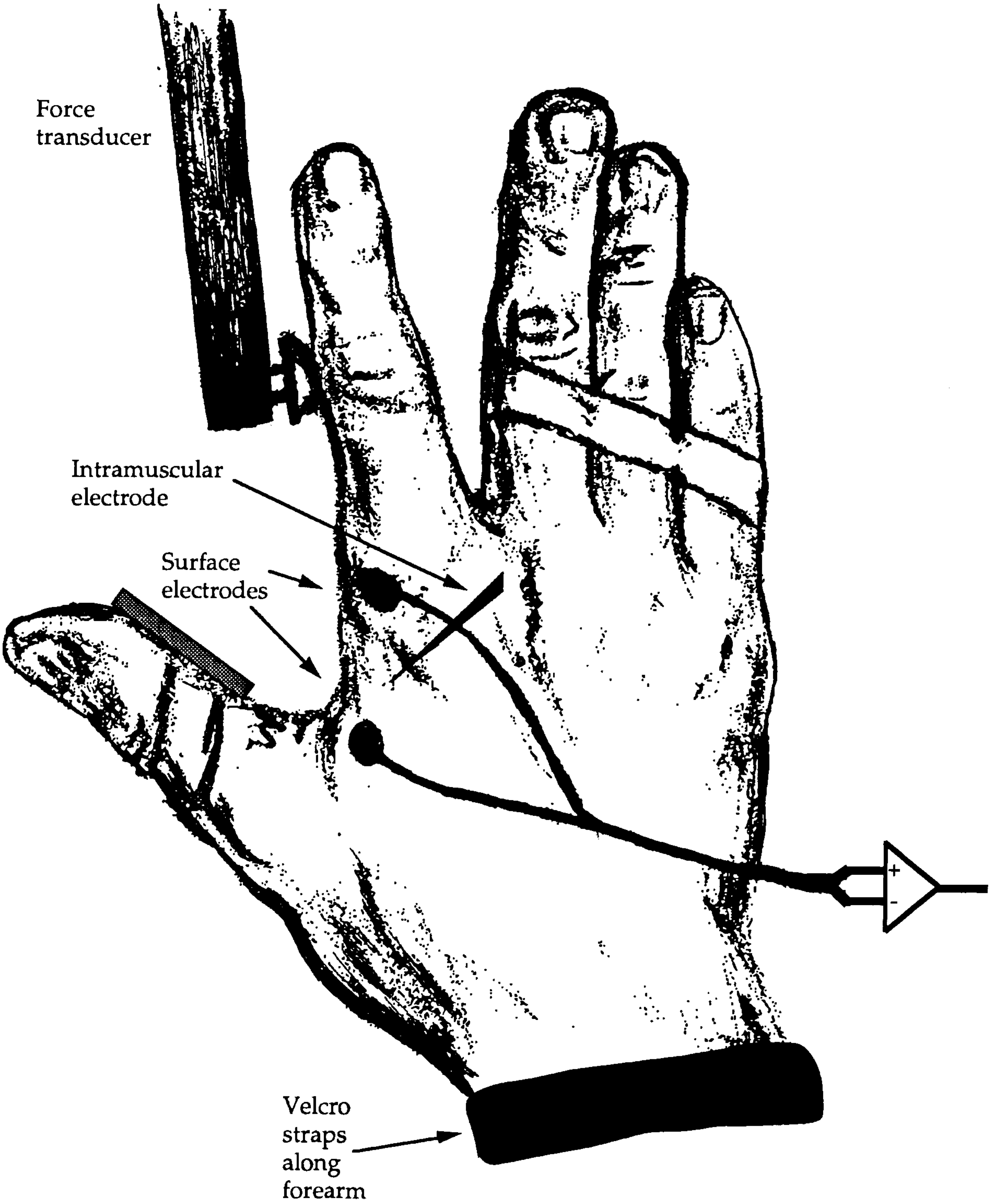
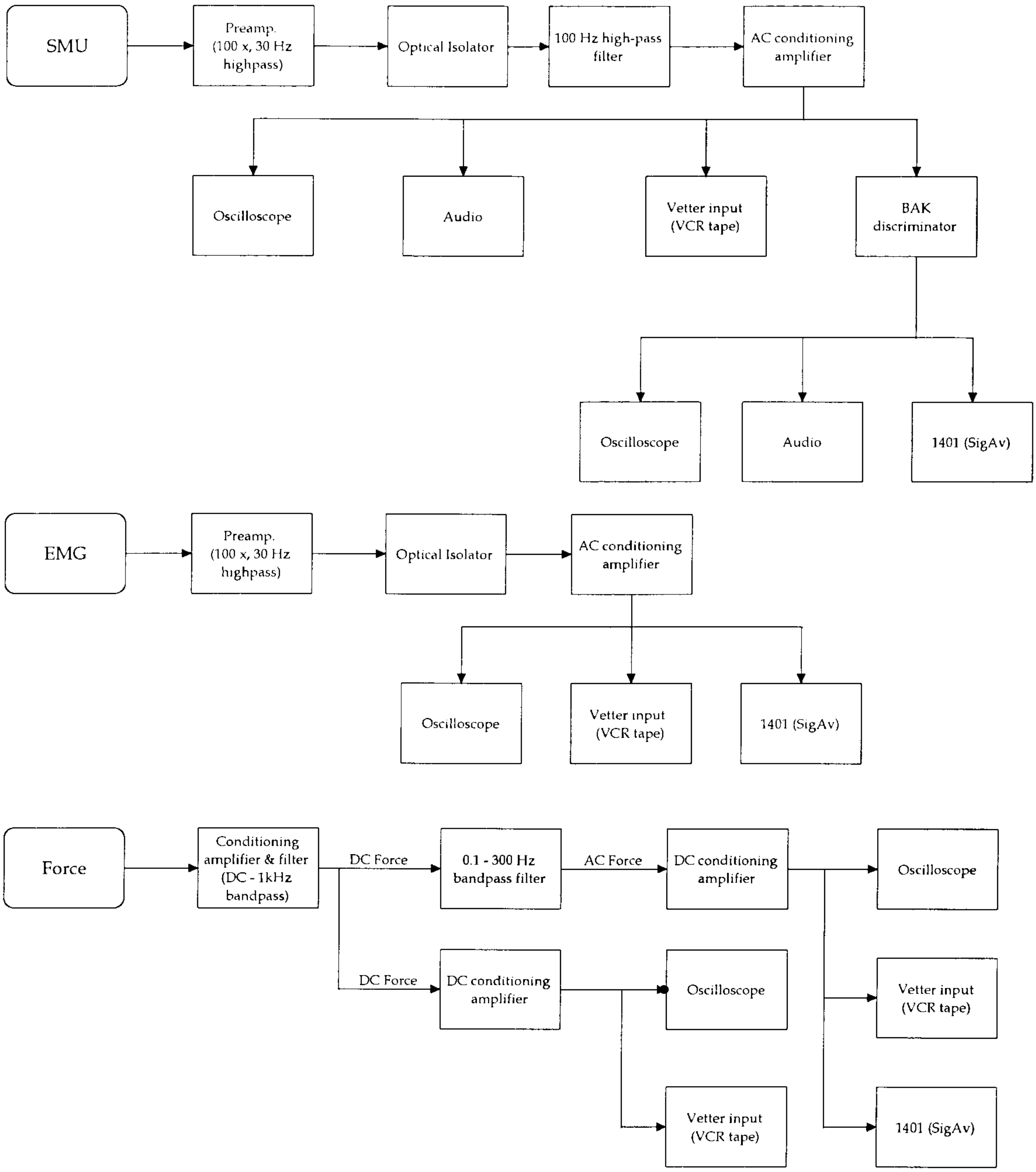

Force
transducer
Intramuscular
electrode
Surface
electrodes
+
-
Velcro
straps
along
forearm

Figure 2.1. (a)



(b)

Figure 2.1. Experimental set-up. (a) electrode positioning on the hand. (b) Schematic of the data collection system.

The SMU signal was recorded for off-line analysis onto a VCR tape using a model 4000A Vetter PCM (pulse code modulator) recording adaptor. Analog inputs to the PCM were multiplexed and stored serially in a digital format on the VCR. During all experiments the PCM was used in a four-channel mode which allowed sampling of the SMU channel at 44 kHz and of the three remaining channels (surface EMG, DC force, and AC force) at 11 kHz.

SMU recordings were monitored throughout the experiment on oscilloscopes. SMU input was also fed into a Grass AM 8 audio monitor (the 3 dB frequency for the low filter was 30 Hz and the 3 dB frequency for the high filter was 15 kHz). As well, the SMU activity was discriminated using a Bak Electronics Inc. (Model DIS-I) discriminator to facilitate tracking of a single motor unit. The discriminated motor unit action potential produced a +4 V pulse (a TTL pulse) for the unit. The TTL pulse was fed into the audio monitor for subject auditory feedback and into an A/D convertor (1401 Plus Analog-to-Digital Convertor: CED; Cambridge, G.B.). The A/D convertor is an interface to a transient signal capture and averaging program (SIGAV: CED; Cambridge, G.B.). SIGAV was run on a 25 MHz 386 PC for on-line spike triggered averaging of the surface EMG and of the AC force with respect to TTL pulses of a motor unit (Stein et al., 1972; Milner-Brown et al., 1973; Thomas, 1995).

2.2.2 Surface EMG

Surface EMG was recorded using a bipolar electrode configuration of two, 9 mm Ag/AgCl disk electrodes on the FDI muscle. One electrode was placed over the belly of the muscle while the second one was over the tendon connecting FDI to the base of the first phalange joint of the index finger (see Figure 1 in Appendix A). Grass Instrument Company Electrode Cream was an interface between the disk electrodes and the subject's skin. A reference ground electrode was wrapped around the subject's right biceps.

As with the SMU, surface EMG was first amplified and passed through a 30 Hz highpass filter in the Neurolog System preamplifier. Next, the signal was conducted, via the Neurolog System isolator, to a conditioning amplifier where it was amplified approximately ten times.

During the experiments the surface EMG was monitored on an oscilloscope and acquired at 10.42 kHz to the computer via the 1401 plus A/D convertor. Spike-trigger averaged EMG (or Motor Unit Triggered Average, MUTA) were generated during the experiment on the 386 PC using SIGAV. MUTA was used online for motor unit identification.

The surface EMG signal was recorded on the VCR using a sampling rate of 11 kHz (via the Vetter PCM recording adaptor) as described in the previous section on Single Motor Unit Activity.

2.2.3 Force

A Grass Force-Displacement Transducer (Model FT03 D) made contact with the lateral surface of the index finger at the joint where the proximal phalange meets the middle phalange (Figure 2.1). The transducer's sensitivity and maximum detectable load were variable and depended upon the set of springs used. Since we needed to extract single motor unit twitch tensions, we selected for use the most sensitive pair of springs possible for our range of forces to allow averaging of small, single unit twitch profiles. As such, transducer stiffness was 1 mm/kg and sensitivity was ±50 mg/mm. The maximum working range was 1 kg.

A Vishay Instruments (Model 2310) bridge amplifier was used to detect force, amplify the force signal 200 times, and filter it with a 1 kHz cut-off low pass filter. The output was separated and went in two routes to produce AC and DC force. DC force is the absolute force whereas AC force is the highpass (0.1 Hz) filtered force. AC force, since it has no DC component, facilitates extraction of single motor-unit twitches.

2.2.4 AC Force

The output of the bridge amplifier was filtered by a bandpass filter (0.1 - 300 Hz) and further amplified by the DC amplifiers as required. The amplifier output, AC force, was recorded on the VCR (sampling rate of 11 kHz) as described in the SMU section. The raw AC force signal was monitored on an oscilloscope during the experiment to ensure proper gains and filtering. The AC force signal was also fed to the A/D convertor for spike triggered averaging by the signal averager program, SIGAV, to generate an online profile of the motor unit twitch.

2.2.5 DC Force

The output of the bridge amplifier was fed to a DC conditioning amplifier and amplified as required. The DC force was also monitored throughout the experiment on an oscilloscope and recorded on the VCR tape (11 kHz sampling rate). Refer back to the SMU section for a description of recording methods. DC force was monitored for absolute force output of the muscle.

2.3 Procedure

Subjects performed three maximum voluntary contractions (MVCs) of approximately three seconds duration with one minute rest between each. The surface EMG for the MVCs was rectified and averaged over half-second intervals every 0.25 seconds. The maximum value was taken as an estimate of the MVC.

Subjects were then asked to execute a slow ramp increase in isometric force of abduction to voluntarily recruit either a low or a relatively high threshold motor unit. A total of three ramps were executed to obtain an average recruitment threshold for the unit in terms of surface EMG. EMG at recruitment was an average of rectified surface EMG 0.25 seconds before and after the unit's TTL pulse at recruitment. Recruitment threshold was then expressed in terms of rectified EMG:

$$\text{recruitment threshold} = (\text{EMG at recruitment}/\ \text{MVC EMG}) \times 100\%$$

If possible, the same motor unit was tracked for both the constant rate and constant force parts of the experiment. Low threshold units were those recruited at less than 10% of the MVC whereas higher threshold units were recruited at greater than 10% of the MVC.

Next, the unit was discriminated and the following steps were performed:

Part I: Constant Rate Protocol

Step 1 **(STA 1)** The unit was fired at the slowest rate possible while on-line spike-triggered averaging of the twitch profile was performed (mean inter-spike interval, ISI, greater than 100 ms). This slow firing was required for one to two minutes.

Step 2 **(constant rate paradigm)** Next, the subject was asked to gradually increase the firing rate by decreasing the ISI to less than 100 ms, a rate that the subject could hold comfortably for 10 minutes. Rate feedback was provided on the audio monitor and the oscilloscope screen.

Step 3 **(STA 2)**: Following ten minutes of constant motor unit firing or once the subject had difficulty maintaining the rate of firing, firing rate was decreased to permit spike-triggered averaging of the twitch profile.

Step 4 **(Rest)**: The subject was asked to rest for at least ten minutes. With these relatively low threshold units (<20% of MVC), we found that 10 minutes provided sufficient rest. This was established in the pilot experiments by (a) subjective perception of the subject and (b) the re-establishment of original recruitment characteristics of the motor units with respect to order of recruitment and recruitment threshold.

Part II

Step 5 **(STA 3)**: The motor unit was fired, as in steps 1 and 3, for spike-triggered averaging to obtain the twitch profile.

Step 6 **(constant force paradigm)**: The subject was asked to maintain a constant force. The force level was set at the DC force where the unit fired with an ISI of <100 ms and was held for 10 minutes if possible or until the motor unit being tracked dropped out. Force feedback was provided on an oscilloscope monitor.

Step 7 **(STA 4)**: The subject was asked to decrease the firing rate to less than 10 impulses per second for spike-triggered averaging of twitch profile.

2.4 Data Acquisition

Data were transferred from the VCR cassette for off-line analysis using a 486 personal computer running SPIKE 2 software from Cambridge Electronic Design. The data transfer process involved demultiplexing and digital to analog reconstruction of the data by the PCM. The four channels (SMU, surface EMG, AC force, and DC force) were appropriately filtered and amplified, and the motor unit being tracked on the SMU channel was discriminated to produce a TTL pulse as was done during the experiment. The data were restricted to ±4 V for input to the 1401 A/D computer interface. The filtering was set for the four channels as shown in Table 2.1.

Table 2.1 Filter settings.

Measured Parameter	**Highpass**	**Lowpass**
SMU	100 Hz	10 kHz
EMG	10 Hz	3 kHz
AC Force	DC	300 Hz
DC Force	DC	100 Hz

The SPIKE 2 data capture program was used to capture data via the 1401 A/D computer interface. Data were sampled at the rates shown in Table 2.2.

Table 2.2 Data sampling rates.

Channel	**Measured Parameter**	**Sampling Rate**
01	TTL	100 Hz
02	SMU	10.42 kHz
03	EMG	10.42 kHz
04	AC Force	101 Hz
05	DC Force	50.1 Hz

2.5 Data Analysis

Following data acquisition, data were inspected using the interactive aspect of the data analysis software package from CED (SPIKE 2) to ensure that discrimination had been successful. SPIKE 2 programming scripts had been written to produce generated parameters: first-order interval histograms, twitch profile, MUTA, power spectra, and average force. Table 2.3 describes each generated parameter extracted from the data. More details are provided in the text that follows.

Table 2.3 Generated Parameters

Parameter	STA	Constant Rate	Constant Force	Aspects
First Order Inter-Spike Interval Histogram	~60 s of data	1st & last 2 min of data.*	1st & last 2 min of data.*	mean, S.D., mode, coefficient of variation, # of spikes used for mean and S.D. calculation (cut off on right side at mode + 60% of mode), # of spikes within the time period.
Twitch Profile	~60 s of data	N/A#	N/A	amplitude (mV and Newtons), contraction time (CT, in ms), 1/2 relaxation time (1/2RT, in ms).
MUTA (of surface EMG)	~60 s of data	1st & last 2 min of data.*	1st & last 2 min of data.*	peak-to-peak amplitude (mV), width (ms), peak-to-peak width (ms).
Rectified MUTA	~60 s of data	1st & last 2 min of data.*	1st & last 2 min of data.*	width (peak-to-peak, in ms), visual inspection for synchronization
DC Force	N/A	1st & last 2 min of data.*	N/A	mean force change (kg) when firing rate was held constant.
EMG Power Spectrum	N/A	1st & last 2 min of data.*	1st & last 2 min of data.*	mean power frequency and total rms power.
Average Rectified EMG	N/A	1st & last 2 min of data.*	1st & last 2 min of data.*	mean surface EMG (mV).

*If < 4 - 5 min of data then 1st and last minute. #N/A = Not applicable.

2.5.1 Histogram

A motoneuron interspike-interval (ISI) varies due to noise in the membrane potential. The noise could be inherent in the motoneuron, or may be due to noise in the synaptic inputs. This noise is reflected in the shape and variability of first-order interval histograms. The question was whether the noise changes with prolonged firing.

Histograms of successive ISIs were generated for periods when spike triggered averaging of twitch profile was done and for sections of the constant rate and constant force parts of the experiment. Histograms generated during steps 1, 3, 5, and 7 (STA periods) were only to correct for very short ISIs so that the twitches did not fuse. Histograms generated at the beginning and the end of steps 2 (constant rate) and 6 (constant force) were for the purpose of comparison of histogram parameters. Therefore, this discussion of histogram parameters applies only to the latter group. If motor unit firing was held at a constant rate for greater than four or five minutes then the first two (initial) and last two (final) minutes of the constant rate segment were used to generate histograms. This allowed for comparison of ISI distribution at the beginning and at the end of the constant rate portion of the experiment. If a constant rate was held for less than four or five minutes then the first and last minutes were used for initial versus final comparison. This same method was used to determine which segments of the constant force portion of the experiment were to be analyzed.

From the first-order interval histograms of ISI distribution, the following descriptors were obtained:

- ISI mode (ms)
- ISI mean (ms)
- ISI standard deviation (S.D., measured in ms)
- Coefficient of Variation (CV = ISI mean/standard deviation)
- Standard error of the mean (SE = S.D./$n^{1/2}$)
- Net Events: the number of events (TTL pulses) used for statistical computation after deleting very long intervals (TTL pulses with ISIs greater than 1.6*mode). See the discussion that follows.
- Total Events: the total number of events within the time period selected (e.g., 2 minutes) which were used to construct the first-order interval histogram.

Not all events were used to calculate the mean and standard deviation because subjects occasionally had difficulty maintaining a constant firing rate. Consequently, there were segments of time between firing where the unit had to be re-recruited. These segments gave long ISIs which would distort the mean and were therefore discarded from the calculations: all events with ISIs greater than 160%*Mode (ms) were discounted (similar to methods of Andreassen and Rosenfalck, 1980).

To allow ISIs greater than 160% of the mode would include ISIs where a spike missed discrimination (Figure 2.2). These long ISIs make up a mini peak in the histogram centered around two times the mode. This second peak shown in Figure 2.2 is exaggerated for illustrative purposes. It was not ignored in the data processing but was eliminated by correcting for wrong discrimination. Errors in discrimination are often a result of changes in SMU spike shape due to the addition or subtraction of muscle fibre electrical activity in the region surrounding the needle electrode. Another possible explanation could be that an adjacent motor unit may have fired in synchrony with the motor unit being tracked. In order to minimize these errors, discrimination was watched closely during data acquisition. Further, the data were inspected following transfer and TTL pulses were added and deleted as necessary wherever the spikes were wrongly discriminated.

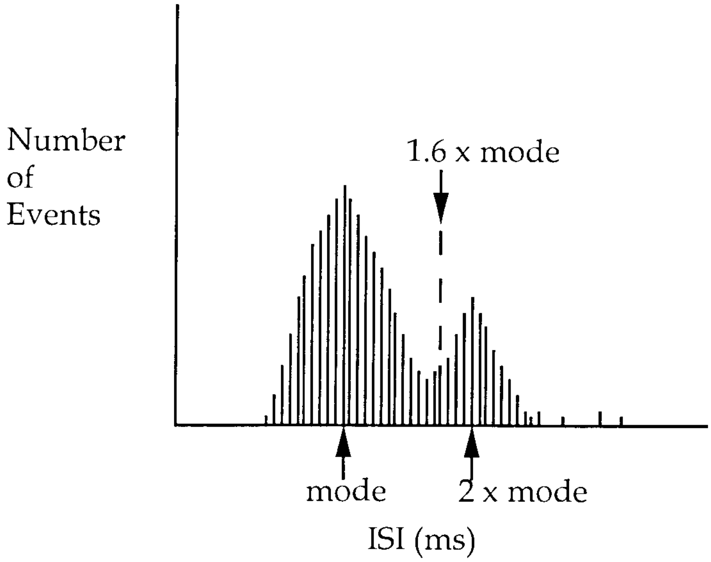


Figure 2.2. First-order interval histogram.

Since only a portion of TTL pulses are used to calculate the mean, the histogram script was written to display the number of events within the 160%*Mode ISI range (net events) in addition to the total number of events within the period of time selected for the histogram analysis (total events).

2.5.2 Twitch Profile

Twitch profile illustrates an aspect of the mechanical properties of a motor unit: it is the impulse force response of a motor unit to an action potential. We generated twitch profile using spike-triggered averaging (Milner-Brown et al., 1973a) of the AC force with the TTL pulses of the motor unit. The magnitude of a twitch profile changes with prolonged firing and could either potentiate or decrease. Twitch profiles were generated before and following the constant rate protocol to permit evaluation of the effect of prolonged activation on muscle unit contractile properties. Twitch profiles were also calculated before and after the constant force protocol. In our experiments, twitch profiles could only be obtained during the STAs when the motor unit was firing slow enough to minimize superposition of subsequent twitches. A maximum firing rate of 10 Hz was necessary to obtain good twitches (Milner-Brown et al., 1973a; see Calancie and Bawa, 1986). Amplitude, contraction time (CT), and 1/2 relaxation time (1/2 RT) were the three descriptors obtained from the twitch profiles (Figure 2.3).

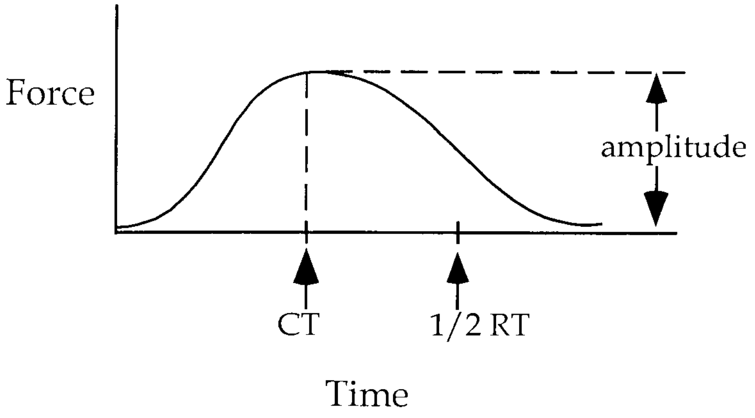


Figure 2.3. Descriptors of motor unit twitch.

2.5.3 MUTA

The motor-unit triggered average, MUTA, is a measure of the almost synchronous electrical activity of all muscle fibres of the motor unit when extracted by spike-triggered averaging of surface EMG activity (Palmer and Fetz, 1985; Lemon et al., 1990). With respect to surface EMG electrodes, the relative current arriving at the surface electrode stays the same, even though the microelectrode recorded shape of a unit may change. The tracking and discrimination of motor units becomes difficult when there are a couple of units with similar SMU shapes. When one of the two units has been discriminated we can distinguish it from the second by the shape of its MUTA. In this way, following a rest, we can further verify that we are tracking the same unit as before the rest. MUTA then acts as the signature of the motor unit while microelectrode recorded activity may be changing in shape and size (Palmer and Fetz, 1985). However, with prolonged firing, either the membrane properties and/or the extracellular ionic balance may change and result in a change in profile of the MUTA.

MUTAs were generated for the same parts of the experiment as were histograms; during steps 1, 3, 4, and 5 as well as for the start and end segments of the constant rate and constant force periods.

From the MUTAs generated, the following descriptors were obtained (Figure 2.4):

- maximum value (mV)
- minimum value (mV)
- time of maximum value, t_{max} (ms)
- time of minimum value, t_{min} (ms)
- peak to peak width (t_{max} - t_{min}, ms)
- width of MUTA (ms)

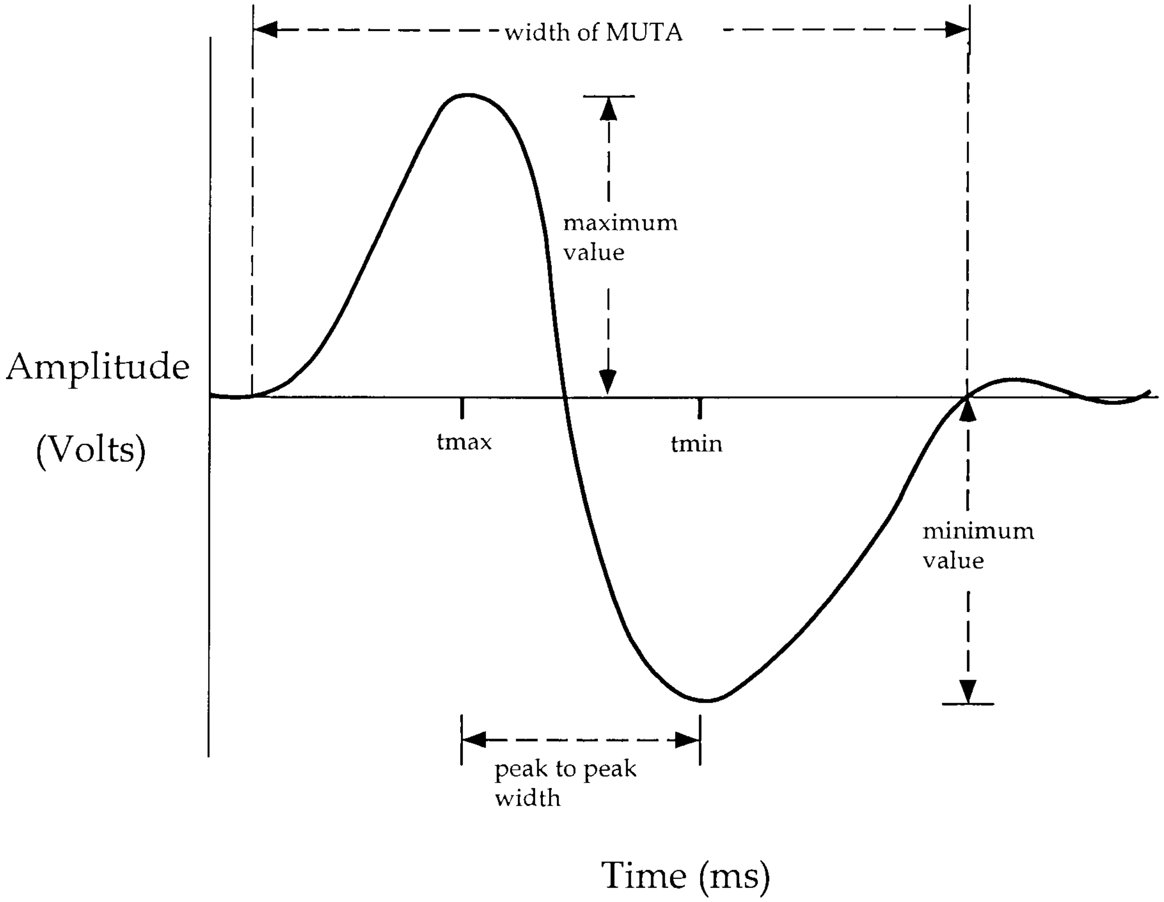


Figure 2.4. MUTA descriptors.

2.5.4 Rectified MUTA

Rectified MUTA was obtained using spike-triggered averaging of the rectified EMG channel. Spike-triggered averaging assumes random (asynchronous) firing of all motor units relative to the discriminated motor unit so that the random effect of all other motor units on surface EMG is cancelled out during the averaging process and only the motor unit being examined is time-locked to the trigger. Synchronization implies that some motor units are time-locked to the discharge time of the discriminated unit. As a result, their surface EMG activity adds to the shape of rectified MUTA, making it larger and/or wider (Figure 2.5). Rectified MUTA was generated to find evidence of synchronous firing of multiple motor units (Milner-Brown et al., 1973a). With increasing fatigue and especially tremor brought on by fatigue, current theory suggests that motor units will fire in synchrony. The EMG channel was rectified using SPIKE 2 analysis software once it had been transferred to the computer.

The following descriptors were obtained to evaluate changes in rectified MUTA (see Figure 2.6):

- max. peak (V)
- 2nd peak (V)
- position of maximum peak, t_{max} (ms)
- position of 2nd peak, t_{2nd} (ms)
- peak to peak width (t_{max} - t_{2nd}, ms)
- width of rectified MUTA (ms)

Rectified MUTA was generated for the same parts of the experiment as was MUTA proper (i.e., steps 1, 3, 5, and 7 to see (a) if there was synchronization during extraction of the twitch, and (b) if synchronization increased as a result of prolonged firing. At the beginning and end of steps 2 and 6 to see if synchronization increased during prolonged firing).

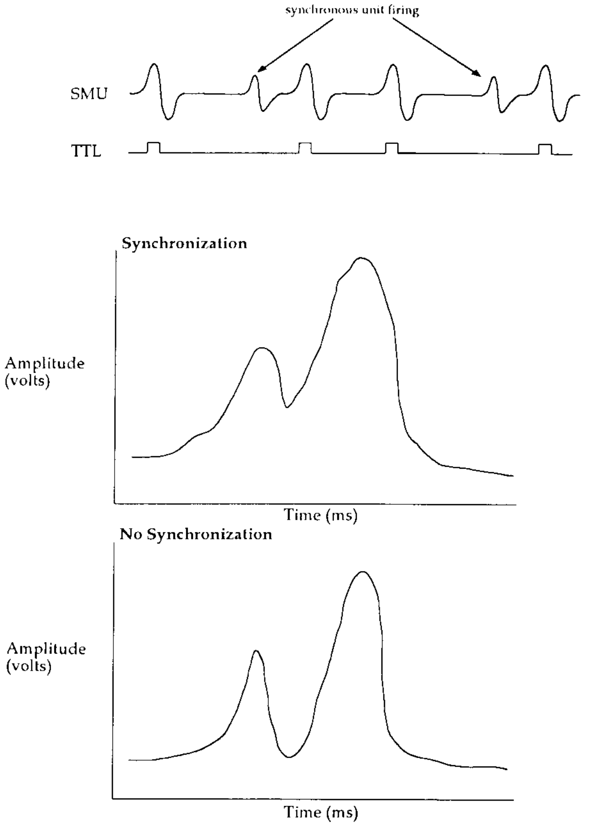


Figure 2.5. Effect of synchronous firing on rectified MUTA.

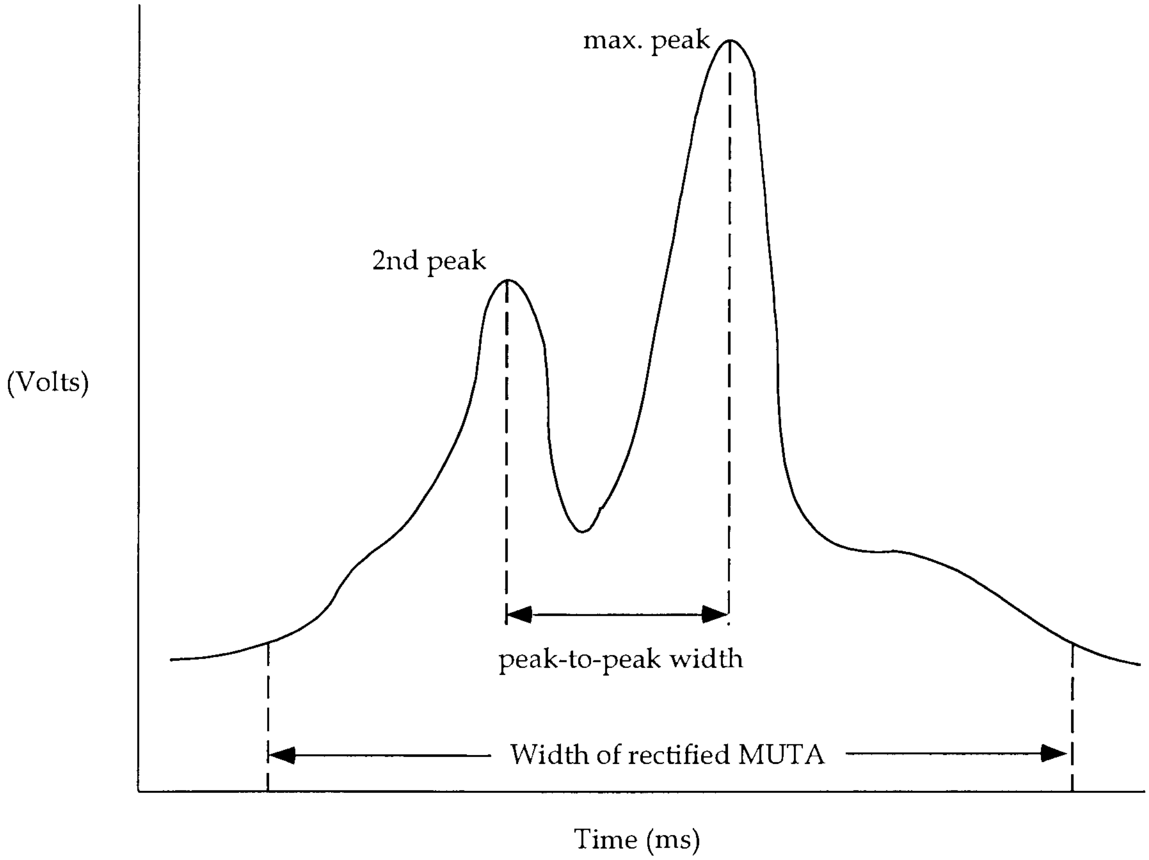


Figure 2.6. Rectified MUTA descriptors.

2.5.5 Force

For constant rate experiments greater than four or five minutes, force was averaged over two-minute blocks at the beginning and end. The two values obtained were compared to see if there was a significant change in force over the course of the experiment. When a subject could not hold a motor unit at a constant rate for more than four minutes then one minute blocks (initial and final) were used for comparison.

2.5.6 EMG Power Spectrum

Changes in the surface EMG frequency power spectrum during fatigue have been reported by numerous researchers (Komi and Tesch, 1979; Moritani et al. 1982; Petrofsky et al., 1982; Krogh-Lund and Jørgensen, 1992; Fuglevand et al., 1993). Surface EMG mean power frequency, median power frequency, and RMS amplitude of the power spectrum have all been used as indices of the frequency content in the EMG signal. Mean power frequency is the frequency of the average power in the EMG power spectrum. Median power frequency, on the other hand, is the frequency at which the spectrum is divided into two regions with equal power. RMS power (root mean square) is a convenient method of expressing power, making calculations of energy simple. In the present study, fatigue has been measured by a decrease in the parameters mean power frequency and median power frequency and an increase in the total RMS power. Mean power frequency and total RMS power were compared for initial and final periods of the constant rate and constant force parts of the experiment to see if there was a change in the power spectrum during prolonged firing. Median power frequency was calculated, but since it provided results similar to those of the mean power frequency, it is not reported in the results section. Mean power frequency is calculated as follows:

$$\text{m.p.f.} = \frac{\sum_{b1}^{b2} (\text{bin\#} * \text{power[bin\#]})}{\text{total RMS power between } t1 \text{ and } t2} * \Delta f .$$

where,

the m.p.f. is calculated within the bin range b1 to b2 where b1 < b2,
bin # = current bin number between (and including) b1 and b2,
power[bin#] = RMS power content of the current bin,

and

Δf = frequency resolution (bin size, Hz).

The SPIKE 2 analysis software automatically scales to RMS power when a power spectrum analysis of a waveform channel is performed using the SETPOWER command. RMS power is calculated as the square root of the power content in the mean spectrum of the channel. EMG data had been sampled by the SPIKE 2 software at 10.42 kHz. Each two or one minute section of data was therefore made up of 1.2504×10^6 or 6.252×10^5 samples respectively. The

SETPOWER function performed FFTs ranging in size from 32 to 4096 points. In the present study, the value of 4096 sample points was chosen to give the highest frequency resolution possible. As such, the frequency resolution, Δf, of the power spectrum was given by,

$$\Delta f = \frac{f_s}{N}$$

where,

N = FFT size (sample points)

and

f_s = sampling frequency.

For a sampling frequency of 10.42 kHz with 4096 sample points, this resulted in a bin size (frequency resolution) of $\Delta f = 2.54$ Hz.

The time domain data were divided into sequential blocks of 4096 points (0.393 seconds long) for FFT processing. Before processing, each block of data was multiplied by a raised cosine window. The reason for multiplication of each block by a raised cosine window was because discontinuity between data at the beginning and end of the block would have distorted the spectrum. The window tapered the two ends of the block to eliminate any discontinuity. SPIKE 2 compensated for the power loss due to the window, but smearing of bins caused by the window could not be avoided. After windowing, an FFT was done on each block of data and then the FFT of all of the blocks were averaged. This process is termed segmental averaging and is used to eliminate noise from the power spectrum.

The generated power spectrum was between 0 and 5.21 kHz (1/2 the sampling rate of the waveform channel) with a frequency increment of 2.54 between each bin.

2.5.7 Rectified Surface EMG

With increasing fatigue, central drive to the motor pool increases (De Luca, 1984; Bigland-Ritchie et al., 1986; Fuglevand et al., 1993). To monitor changes in the central drive we measured mean rectified surface EMG of the initial and final portions of constant rate and constant force (Table 2.3). Channel rectification was done off line using the msf function of the SPIKE 2 analysis software.

An increase in mean EMG of the final period indicates an increase in the central drive to the muscle in response to fatigue.

2.6 Statistics

Comparisons between descriptors of the generated parameters were made using a two-tailed, paired t-test. Changes in parameter value due to fatigue or prolonged firing were compared for the following:

- STA1 versus STA2
- initial versus final periods for both constant rate and constant force conditions
- STA3 versus STA4

Significant difference was reported at the level of $p \leq 0.05$.

3.0 RESULTS

Data are presented for 17 single motor units recorded from five subjects. Results for Part I (constant rate) are from 16 motor units. Twelve of the 16 units were tracked through to Part II (constant force) of the experiment and an additional unit, tracked only for Part II, brought the total number of units tracked during Part II to 13. All 13 units were used for comparisons during STA periods before and after constant force although, for some recordings, a constant force could only be maintained for such a short period of time (that is, less than four minutes) that comparisons between the start and end were impossible. Consequently, only 10 of the 13 motor units could be used for comparisons at the start and end of the constant force procedure.

Motor unit recruitment thresholds for 15 of the 17 units were obtained by the methods described in Chapter 2. Figure 3.1 shows recruitment threshold expressed as a percentage of maximum rectified EMG for motor units arbitrarily numbered 1 to 15. Low threshold units were considered to be those with a recruitment threshold of less than or equal to 10% of the maximum voluntary contraction surface electromyogram (MVC EMG). High threshold units are all units with a recruitment threshold greater than 10% MVC EMG. Five motor units were classified as low, with thresholds ranging from 2.8 to 5.1% MVC EMG. From a total of 15 motor units, ten were high threshold units which were recruited between 12.0 and 70.5% MVC EMG. A recruitment threshold of 70% MVC EMG appears high when compared to recruitment thresholds expressed as a percentage of maximum contraction force; however, this level is realistic given that % MVC EMG is not equivalent to % MVC force. Consider the following, an EMG is due to the interference pattern generated by the firing of motor units within a muscle. A rectified EMG signal may be closely linear until all motor units are recruited at which point (when rate coding is the dominant mechanism of force generation), rectified EMG will plateau.

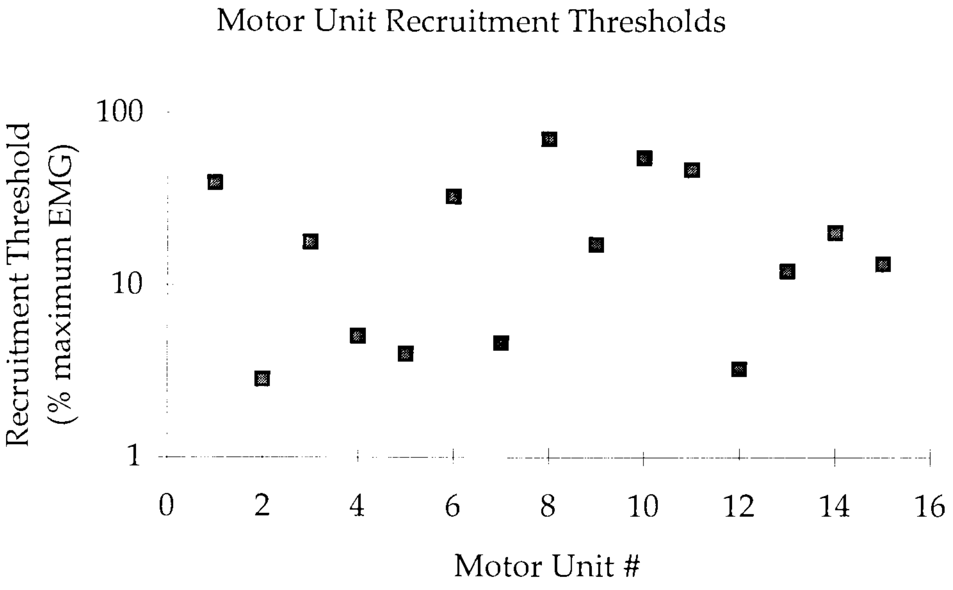


Figure 3.1. Motor unit recruitment thresholds for 15 of 17 motor units examined throughout the experiments. Recruitment threshold is expressed as a percentage of rectified electromyogram (EMG) at maximum voluntary contraction (MVC).

Results that follow will be presented first for Part I, the constant rate section of the experiment, followed by Part II, constant force. For convenience, the experimental protocols are illustrated in Figure 3.2. As described in the previous chapters, the components of the neuromuscular control system which were the subject of this study were the motoneuron, central drive, muscle fibres (their membrane properties), and the force generating system. Evaluation techniques such as first-order interval histograms, spike triggered averaging of EMG and force, and spectral analysis of EMG, were used to monitor changes in motoneuron properties, central drive, muscle fibre membrane properties, and force generation during the prescribed tasks (Figure 3.2). Comparisons were made between neuromuscular system properties before and after the constant rate task (STA 1 versus STA 2), at the start and end of the constant rate task, before and after the constant force task (STA 3 versus STA 4), and at the start and end of the constant force task. Much of the data presented are normalized comparisons. Significance is attained at $p \leq 0.05$ for the two tailed student's t-test.

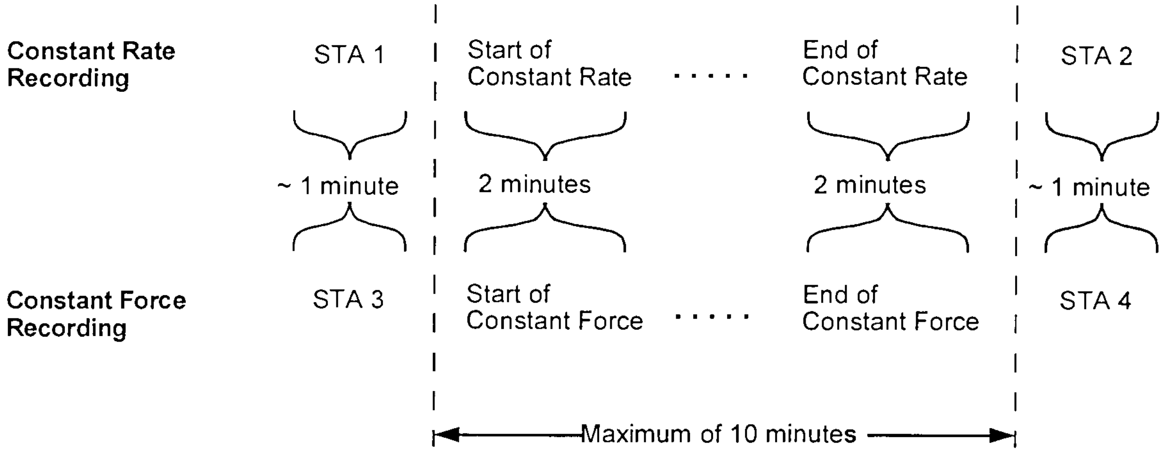


Figure 3.2. The experimental design.

3.1 Part I: Constant Rate Paradigm

3.1.1 Firing Rate of the Single Motor Unit

16 single motor units were discharged for ten minutes, excluding the STA periods. The mean firing rates of the 16 units ranged from 7.2 impulses per second to 13.1 impulses per second (mean ± standard deviation for all units = 9.6 ± 1.7 Hz) during the first two minutes of the constant rate period (or one minute if the recording was less than four minutes). At the end of this firing period, mean rates ranged from 7.6 to 14.7 impulses per second (mean ± standard deviation for all units = 10.6 ± 2.2 Hz). Figure 3.3 (a) and (b) illustrates the first order interval histogram for a single motor unit firing at the start and at the end of the period of constant firing. Comparisons of the same unit's inter-spike interval parameters, mean firing rate, mode firing rate, and the coefficient of variation of firing (a measure of variability), are illustrated in Figure 3.3 (c). For this unit, both the mean and the mode inter-spike intervals (ISI) decreased, corresponding to an increase in firing rate. At the same time, the coefficient of variation was virtually unchanged between the start and the end of constant firing, meaning there was no change in firing variability as a result of prolonged firing. Cumulative results were calculated for the 16 motor units and are shown in Figure 3.3 (d). For the group, both the mean and the mode inter-spike intervals decreased by 10 ± 2% (mean ISI: $p = 0.009$; and mode: ISI $p = 0.01$), corresponding to an increase in the overall rate of firing. Averaging among the 16 motor units, the coefficient of variation remained virtually unchanged.

Although the intention of this portion of the experiment was to maintain a constant firing rate, these results show a small increase in mean firing rate within the group of motor units. More significant, the constant rate portion of the experiment showed that when a relatively constant firing rate is maintained for a prolonged period of time, firing rate variability remains constant. This result is contrary to what was expected because, in the literature, firing rate variability had been reported to increase with fatigue.

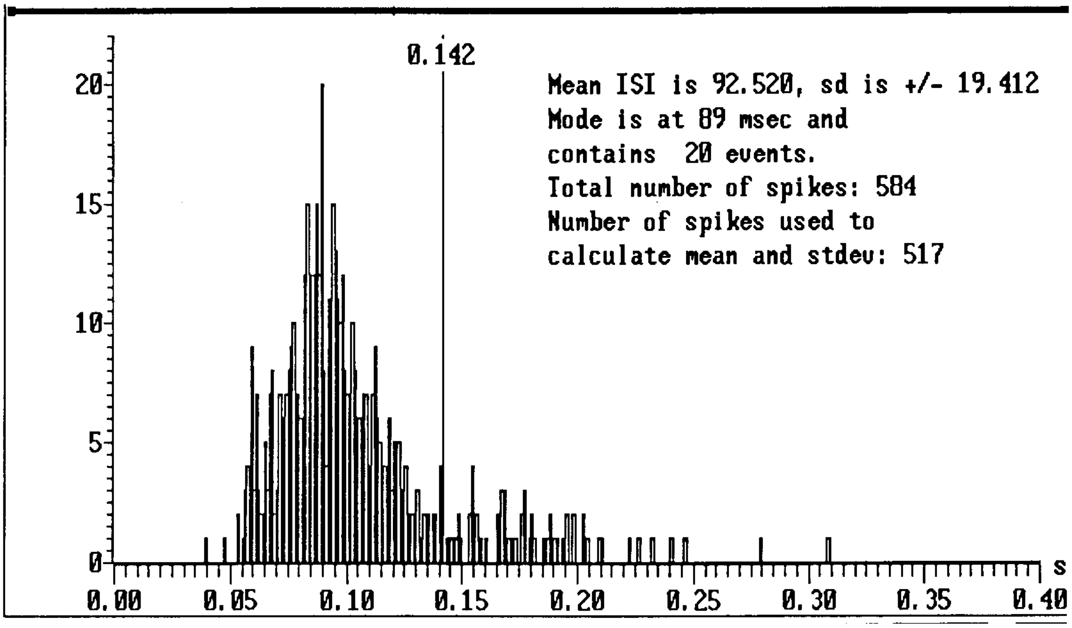


Figure 3.3 (a)

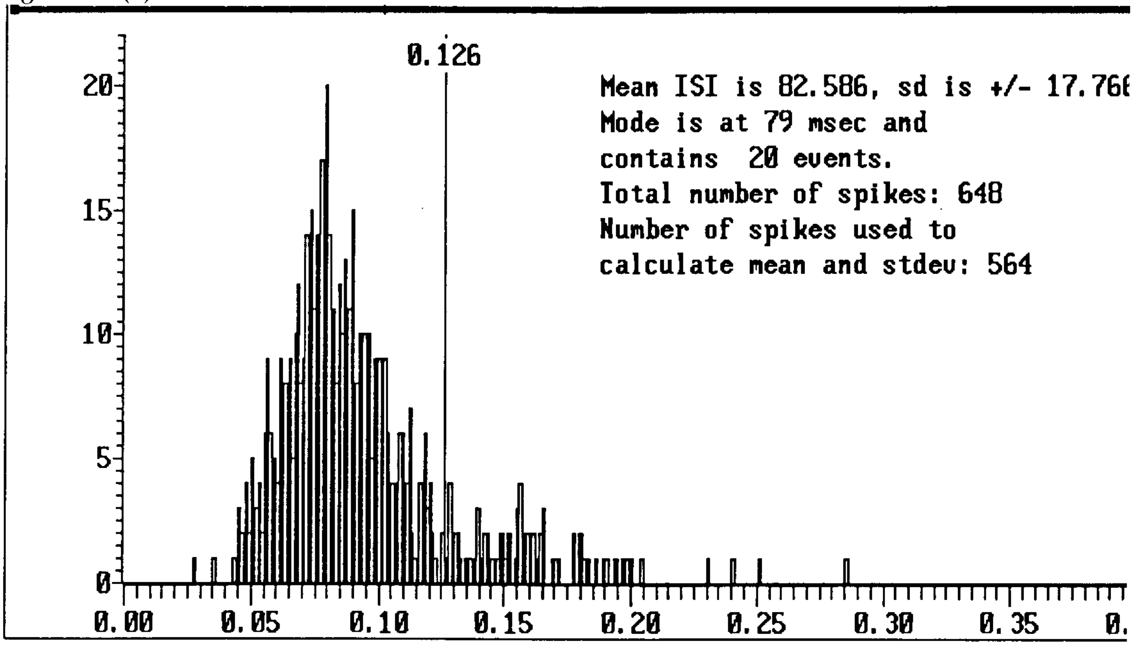


Figure 3.3 (b)

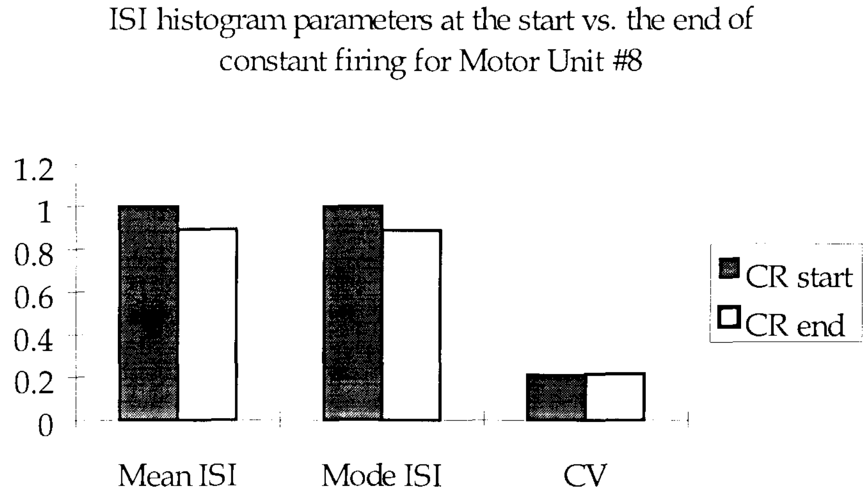


Figure 3.3 (c)

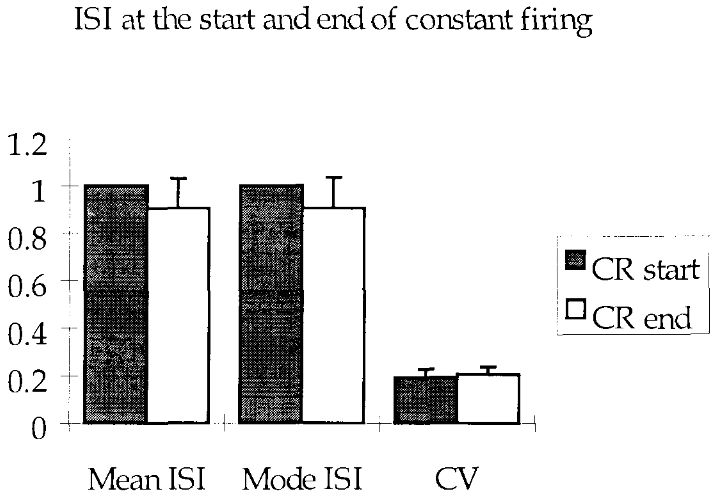


Figure 3.3 (d)

Figure 3.3. Variability of motor unit firing rate at the start versus the end of an extended period of constant firing. For brevity, the constant rate period is abbreviated by CR. Two parameters, mean and mode inter-spike interval (ISI), were used to monitor the consistency of firing rate while the coefficient of variation (CV = standard deviation/mean) monitors the variability of motor unit firing. Part (a) illustrates a first order interval histogram of ISI at the start of CR firing for motor unit 8. Part (b) illustrates a first order interval histogram of ISI at the end of the CR period for motor unit 8. Part (c) provides a comparison mean and mode firing rate as well as coefficient of variation measured from the histograms in (a) and (b). Mean and mode ISIs are normalized to start values for comparative purposes. Part (d) is similar to (c) but illustrates the overall effect of prolonged firing on mean and mode ISI and coefficient of variation for n = 16 motor units.

Comparisons were also made within high and low threshold unit groups. Neither group showed a significant increase (for significance, $p \leq 0.05$, two-tailed student's t-test) in the coefficient of variation between the start and the end of the constant rate period of firing.

3.1.2 EMG

To examine changes in central drive to the motoneuron pool, average rectified surface EMG was computed for two minute blocks[5] at the start and end of ten minutes of constant firing. Figure 3.4 illustrates the change in rectified EMG from start to end. Part (a) of the figure shows the effect of prolonged firing on average rectified surface EMG while motor unit #8 was being tracked. As was expected, rectified EMG increased with prolonged firing. Identical results were obtained for the group of sixteen motor units: a significant increase ($p = 0.006$) was seen in average rectified EMG from the start to the end of the constant rate period (Figure 3.4, part b). An increase in rectified EMG is evidence of an increasing drive to the motor unit pool. Comparisons were also made between high and low threshold units but no significant difference in results was obtained.

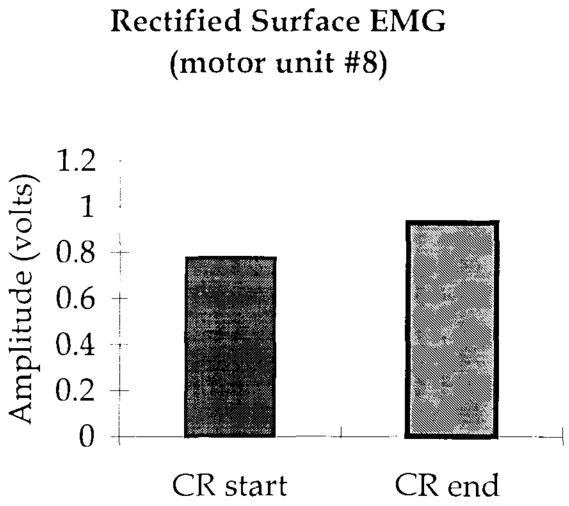
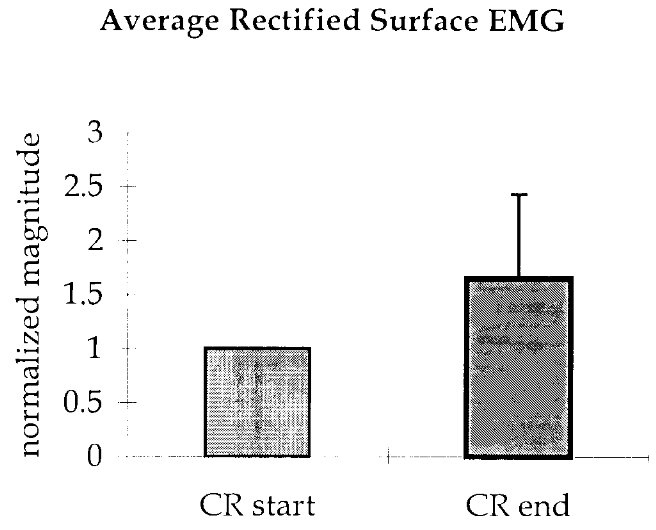

Rectified Surface EMG
(motor unit #8)

Figure 3.4 (a)

---

[5] Two minute blocks were used for constant rate recordings greater than four minutes. One minute blocks were used for recordings of less than four minutes.



Average Rectified Surface EMG

Figure 3.4 (b)

Figure 3.4. Rectified EMG change between the start and end of a period of constant motor unit firing. EMG is averaged over a period of one minute for recordings of less than four minutes total or over two minutes for recordings of four to ten minutes. (a) rectified EMG taken from the subject from whom motor unit #8 was recorded. Amplitude is measured in volts. (b) average rectified EMG changes averaged for all motor units (n=16). Error bar indicates standard deviation.

3.1.3 MUTA

To investigate changes in muscle membrane properties, single motor unit MUTAs were extracted from STA recordings just prior to and following the period of constant firing (STA 1 versus STA 2) as well as at the start and end of the period (refer back to Figure 3.2). Two parameters were used to monitor change in MUTA: peak-to-peak amplitude and peak-to-peak width. For the group of 16 motor units, comparisons between STA 1 and STA 2 showed that MUTA peak-to-peak amplitude increased significantly ($p = 0.02$) whereas peak-to-peak width did not ($p = 0.26$). Figure 3.5 illustrates the change in MUTA amplitude and width between STA 1 and STA 2.

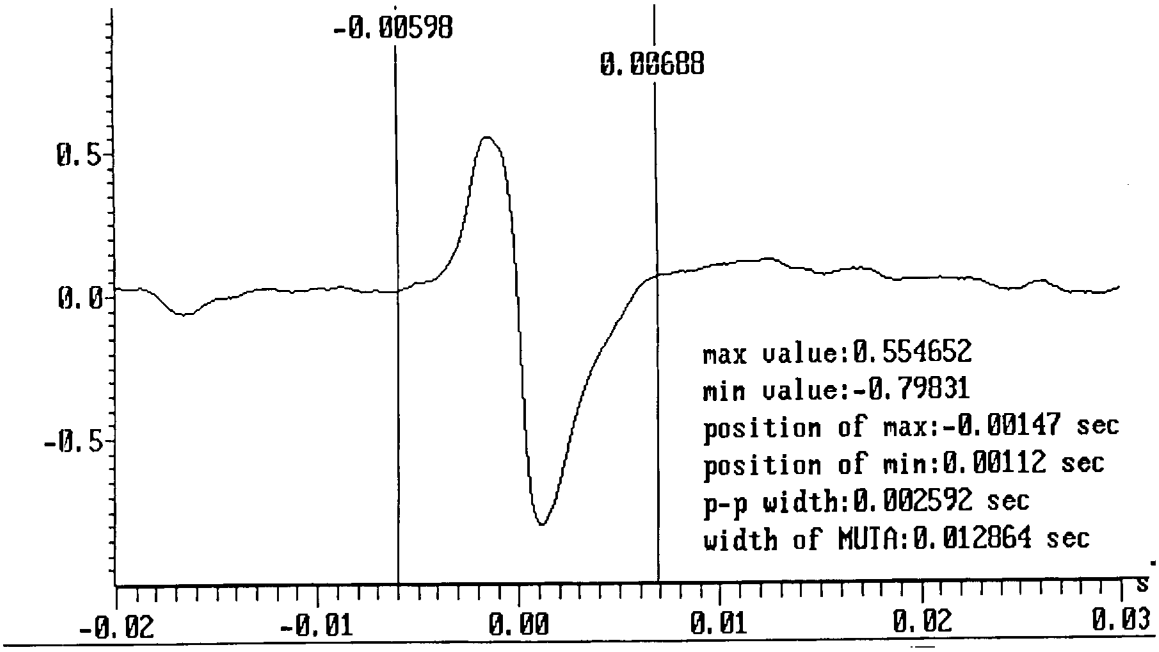


Figure 3.5 (a)

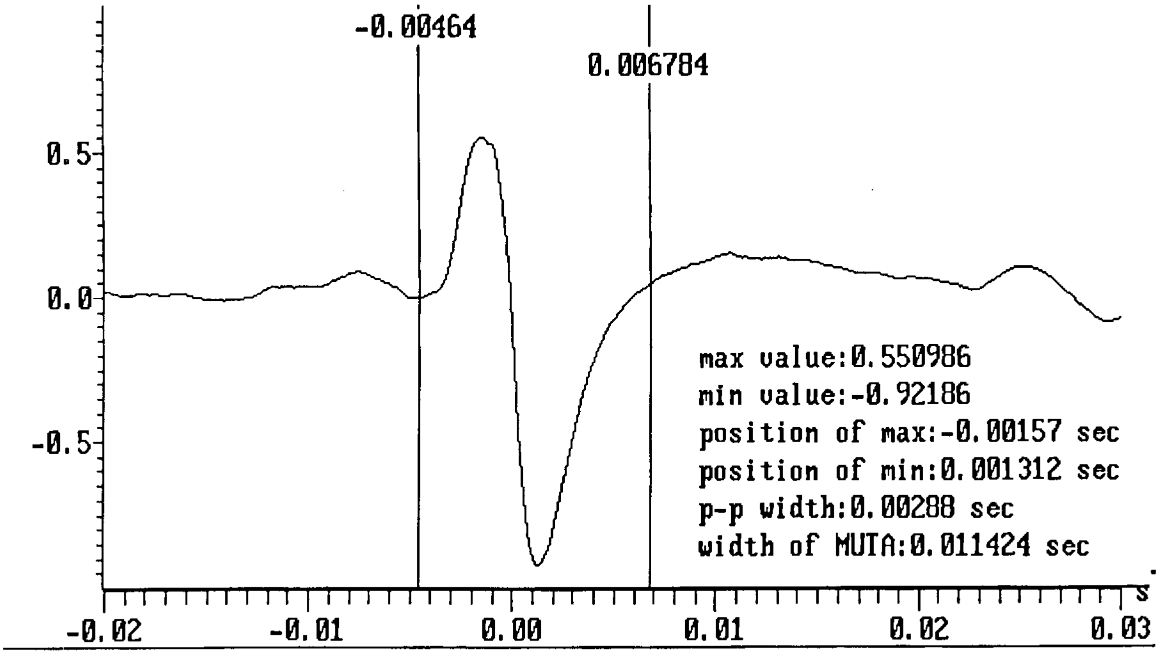


Figure 3.5 (b)

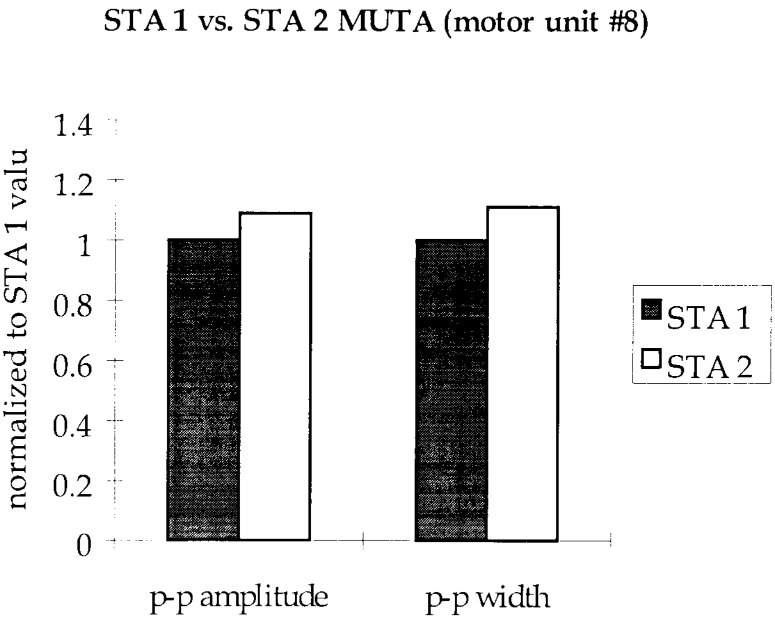


Figure 3.5 (c)

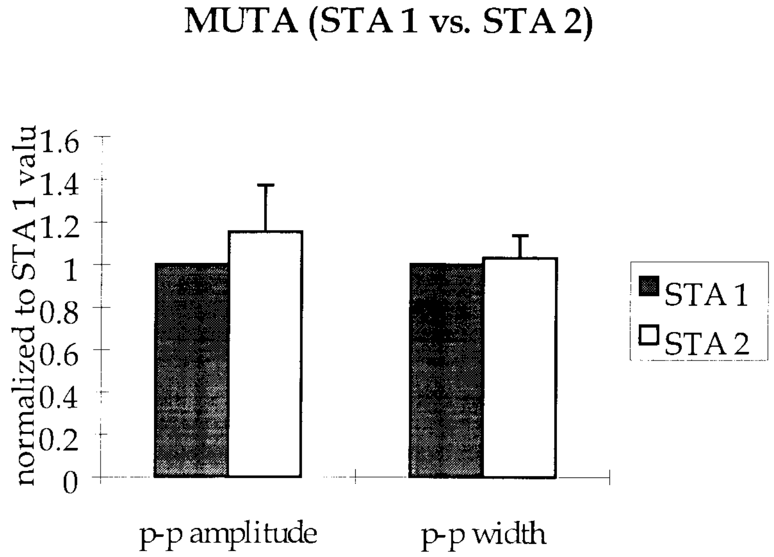


Figure 3.5 (d)

Figure 3.5. MUTA peak-to-peak amplitude and peak-to-peak width compared between STA 1 and STA 2. (a) MUTA for motor unit #8 obtained during STA 1. (b) MUTA for motor unit #8 obtained during STA 2. (c) Direct comparison of peak-to peak amplitude and width between STA 1 and STA 2 for motor unit #8. For comparison, values were normalized to values measured for STA 1. (d) Comparison between STA 1 and STA 2 of MUTA amplitude and width for n=16 motor units. Error bars denote standard deviation.

MUTA peak-to-peak amplitude measured at the start and the end of constant firing also increased but this increase did not attain significance (p = 0.12). Similarly, the change in MUTA width was also insignificant (p = 0.38). Figure 3.6 illustrates the change in MUTA amplitude and width for the group of sixteen motor units.

To see if there were any differences in the behaviour of high versus low threshold units, similar comparisons were made within the high and low threshold groups (Of the 16 motor units used for Part I of the experiment, 5 were low threshold units, 9 were high threshold units, and 2 units had unknown thresholds). No differences were seen between high and low threshold units as monitored by MUTA peak-to-peak amplitude and width measured between STA 1 and STA 2 or between the start and end of the period of constant firing.

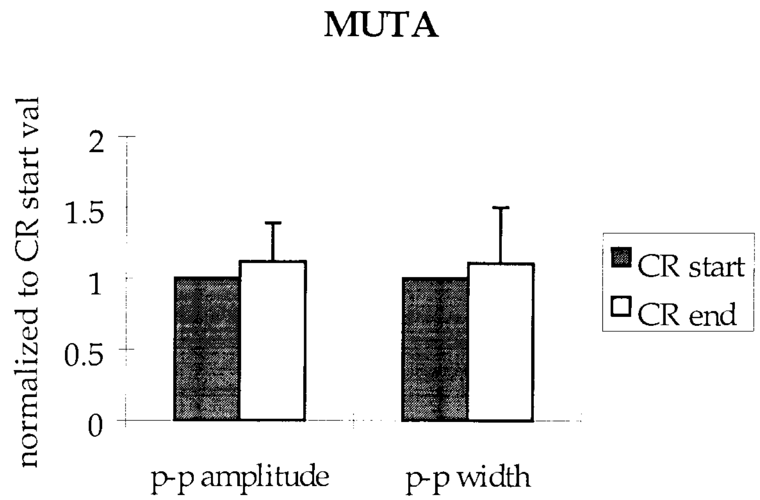
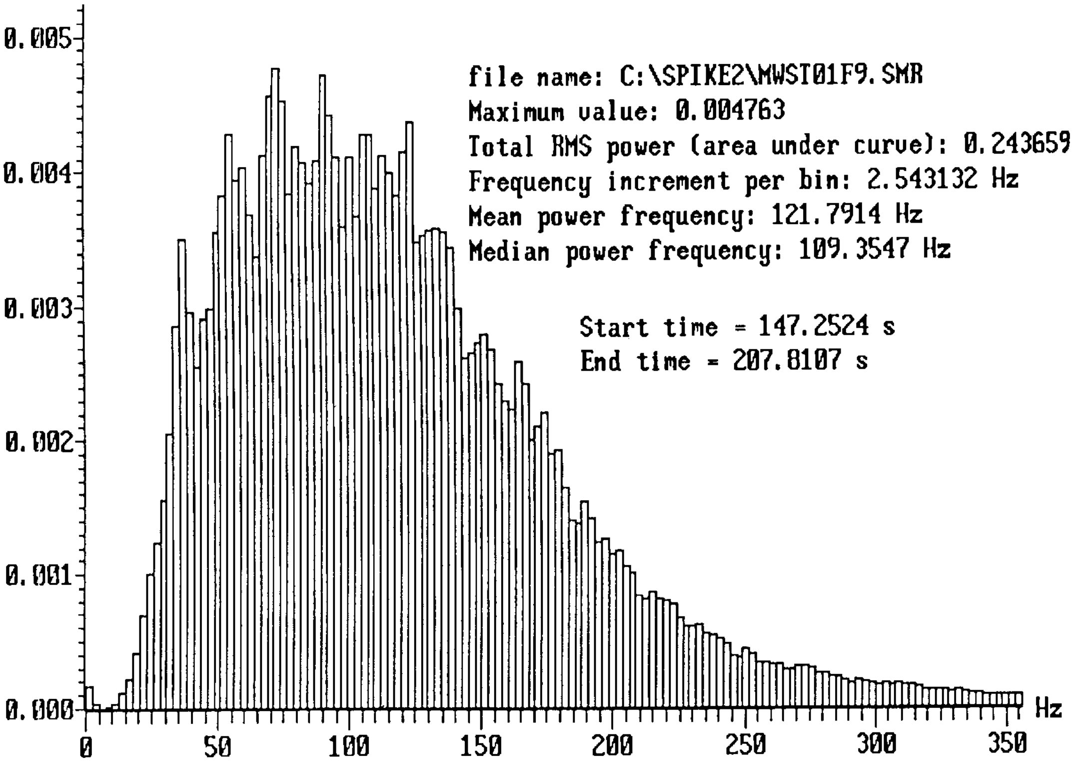


Figure 3.6. Comparison between MUTA parameters peak-to-peak amplitude and width measured at the start and end of the period of constant firing. The values shown on the graph are normalized to the values measured at the start of the period of constant rate. n = 16 motor units. Error bars denote standard deviation.

3.1.4 EMG Frequency Spectrum

The frequency spectrum of surface EMG is affected by a variety of factors which include the following: muscle membrane properties, motor unit type, and firing rate of the contributing motor units. One or all of these factors could change during prolonged firing. Surface EMG power spectra were compared during the STA (Figure 3.7) and at the start and end of the constant firing period (Figure 3.8). Two parameters were measured and compared from the spectra, RMS power and mean power frequency. RMS power is a measurement similar to that done in section 3.1.2 where central drive to the motoneuron pool was monitored by rectified EMG measurements. As such, RMS power may also be used to measure changes in drive to the motoneuron pool. The second parameter, mean power frequency, is the frequency of the average power in the EMG power spectrum. This parameter is used to measure a shift in the dominant frequencies of the spectrum.

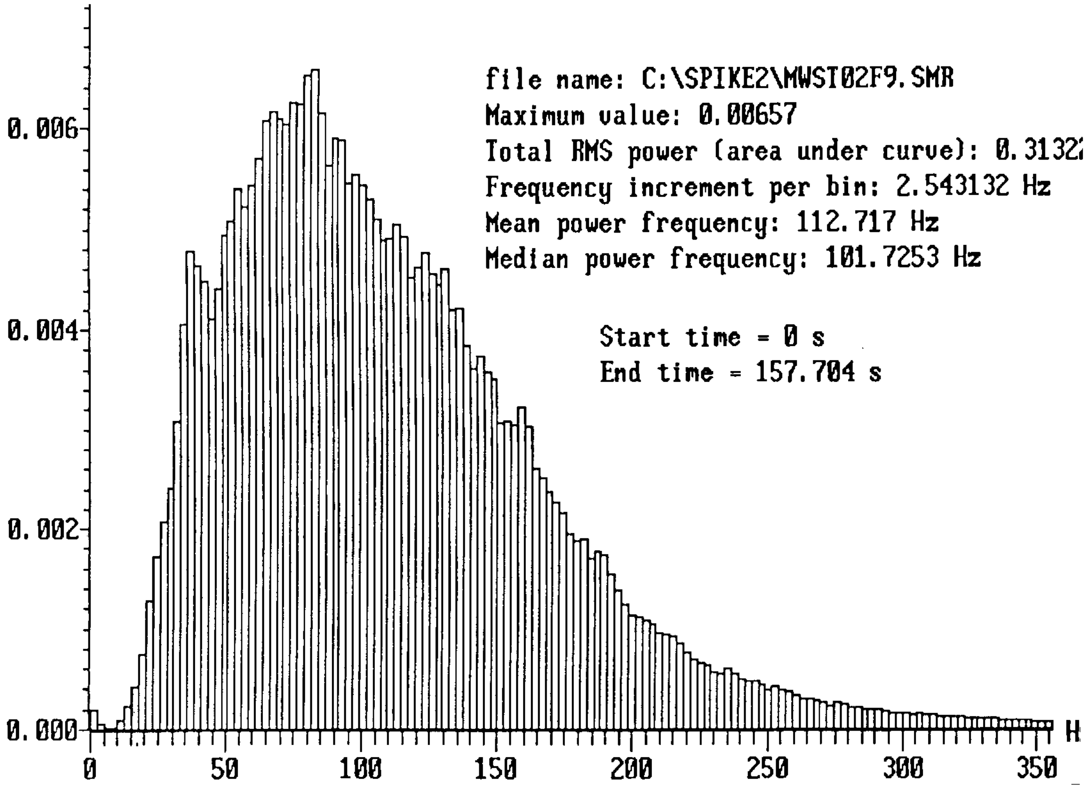
As expected, RMS power increased significantly both between STA 1 and STA 2 ($p = 0.005$) and from the start to the end of the period of constant firing ($p = 0.01$). Changes in mean power frequency between STA 1 and STA 2 were inconsistent ($p = 0.49$): Figure 3.7 (c) shows the mean power frequency dropping for motor unit #8 while Figure 3.7 (d) shows an unchanging mean power frequency. Between the start and end of the period of constant firing (Figure 3.8), the frequency dropped but this drop did not attain significance ($p = 0.24$). Comparisons made within the smaller sub-groups of low and high threshold units failed to produce any evidence of behaviours characteristic of those groups.



```
file name: C:\SPIKE2\MWST01F9.SMR
Maximum value: 0.004763
Total RMS power (area under curve): 0.243659
Frequency increment per bin: 2.543132 Hz
Mean power frequency: 121.7914 Hz
Median power frequency: 109.3547 Hz

Start time = 147.2524 s
End time = 207.8107 s
```

Figure 3.7 (a)



```
file name: C:\SPIKE2\MWST02F9.SMR
Maximum value: 0.00657
Total RMS power (area under curve): 0.3132
Frequency increment per bin: 2.543132 Hz
Mean power frequency: 112.717 Hz
Median power frequency: 101.7253 Hz

Start time = 0 s
End time = 157.704 s
```

Figure 3.7 (b)

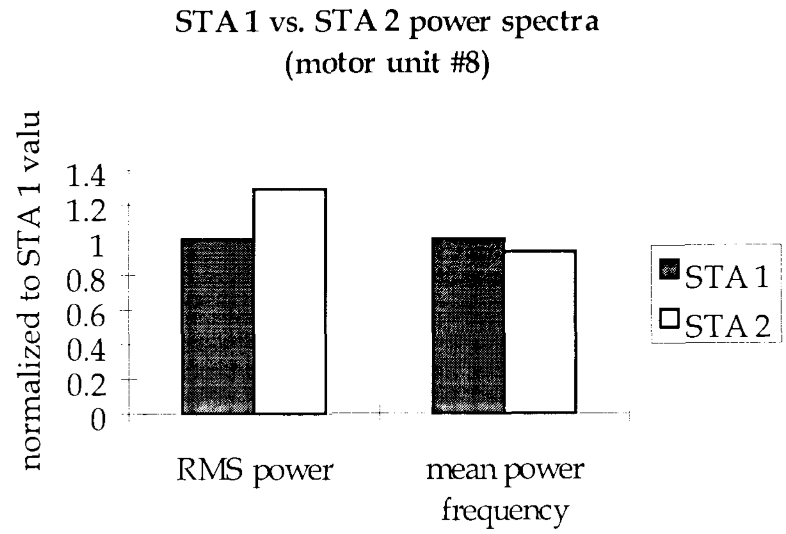


Figure 3.7 (c)

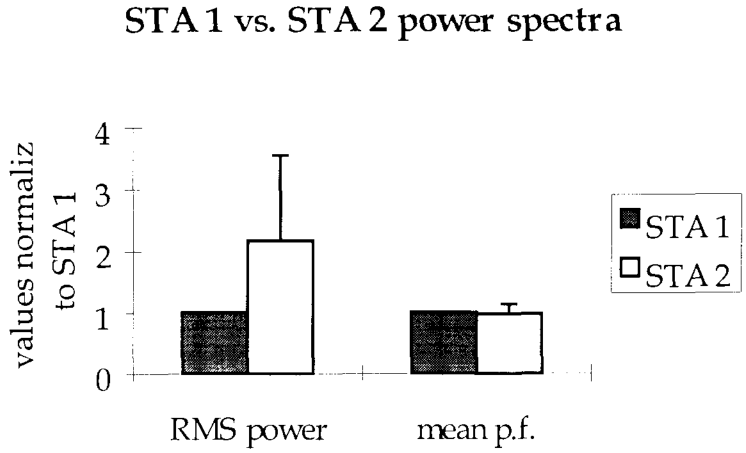
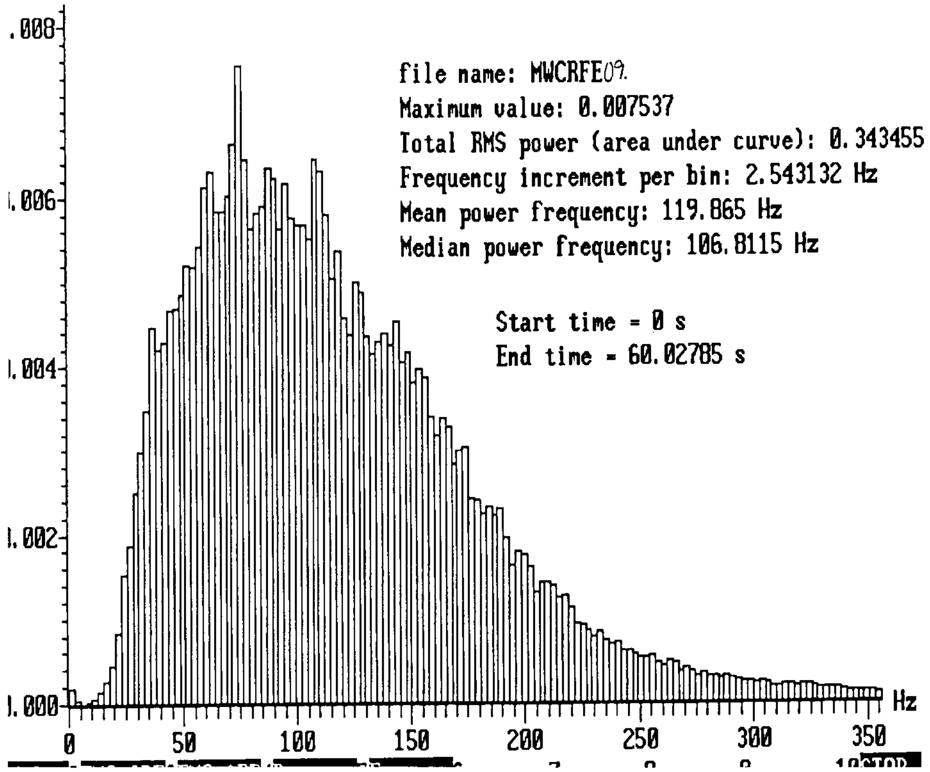


Figure 3.7 (d)

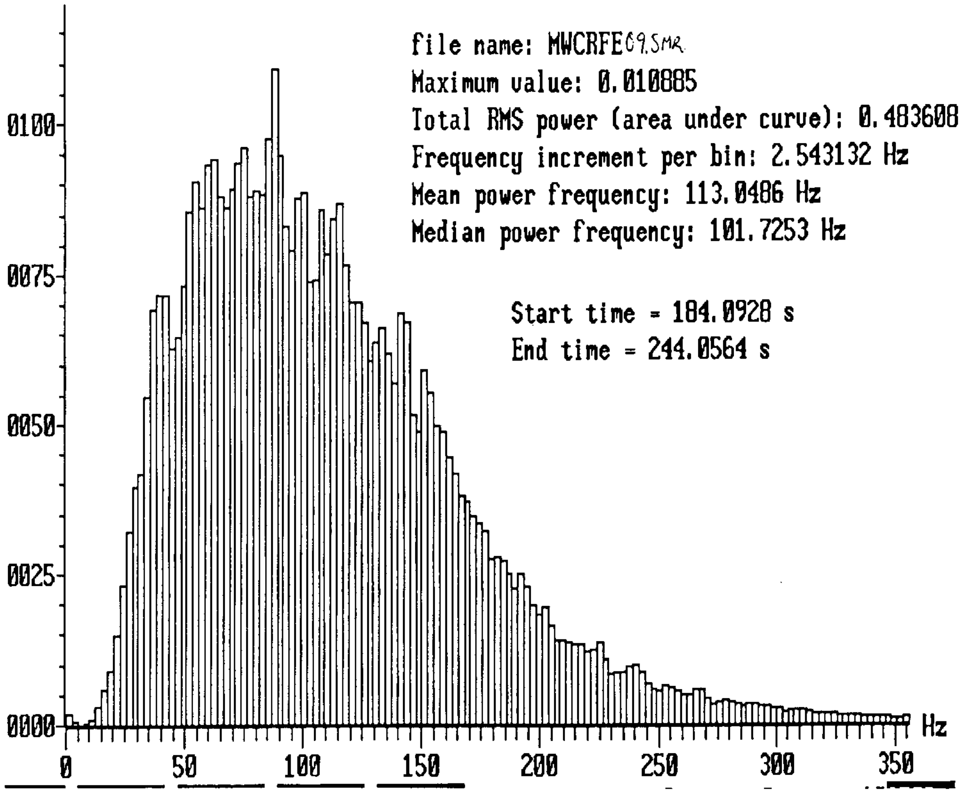
Figure 3.7. Power spectra of STA 1 versus STA 2. Changes are measured by the parameters RMS power and mean power frequency. (a) Power spectrum taken during STA 1 for motor unit #8. (b) Power spectrum taken during STA 2 for motor unit #8. (c) Comparison between STA 1 and STA 2 for RMS power and mean power frequency which were recorded when motor unit #8 was being tracked. Values are normalized to STA 1 measured values. (d) Comparison of RMS power and mean power frequency between STA 1 and STA 2 for the group of n=16 subjects. Values are normalized to values measured during STA 1. Error bars denote standard deviation.



file name: MWCRFE09.
Maximum value: 0.007537
Total RMS power (area under curve): 0.343455
Frequency increment per bin: 2.543132 Hz
Mean power frequency: 119.865 Hz
Median power frequency: 106.8115 Hz

Start time = 0 s
End time = 60.02785 s

Figure 3.8 (a)



file name: MWCRFE09.SMR
Maximum value: 0.010885
Total RMS power (area under curve): 0.483608
Frequency increment per bin: 2.543132 Hz
Mean power frequency: 113.0486 Hz
Median power frequency: 101.7253 Hz

Start time = 184.0928 s
End time = 244.0564 s

Figure 3.8 (b)

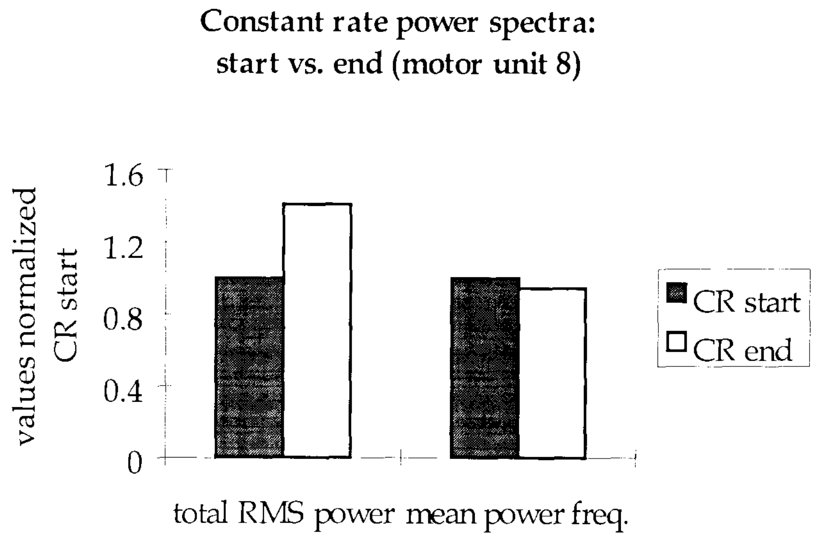


Figure 3.8 (c)

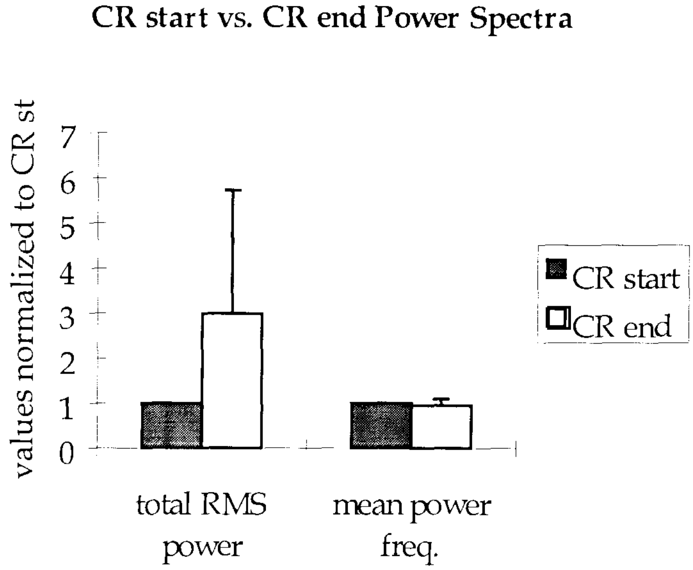


Figure 3.8 (d)

Figure 3.8. Comparison of power spectra taken at the start of the period of constant firing versus power spectra taken at the end. (a) Power spectrum taken at the start of the period of constant firing recorded when motor unit #8 was being tracked. (b) Power spectrum taken at the end of the period of constant firing while motor unit #8 was being tracked. (c) A comparison of the constant rate start versus end power spectra parameters RMS power and mean power frequency which were recorded while motor unit #8 was being tracked. Values are normalized to start values. (d) A comparison between constant rate start and constant rate end for the parameters RMS power and mean power frequency for the group of n=16 subjects. Values are normalized to values measured at the start of the constant rate period. Error bars denote standard deviation.

3.1.5 Twitch Profile

Motor unit twitch profiles were computed prior to and following ten minutes of constant firing (STA 1 and STA 2, refer to Figure 3.2). Three parameters were obtained from each twitch profile: amplitude, contraction time, and 1/2 relaxation time (see Chapter 2 for a description of these parameters). These twitch parameters, which reflect the contractile properties of the force generating system, will potentiate, stay the same, or attenuate following a fatiguing activity. In this case, a significant potentiation ($p = 0.01$) of the twitch profile was seen for all units as a group from STA 1 to STA 2 (see Figure 3.9). Figure 3.9 (a, b, and c) also illustrates the potentiation in twitch profile for a single motor unit, motor unit #8. The unit showed a 46% increase in twitch amplitude from STA 1 to STA 2.

Twitch contraction time increased for many units and the group effect seems to indicate an overall increase in contraction time without having attained significance ($p = 0.09$). On the other hand, some units, such as motor unit #8, were unchanged prior to and following constant firing (Figure 3.9). The group trend for 1/2 relaxation time seems to show a decrease but it too was not significant ($p = 0.07$).

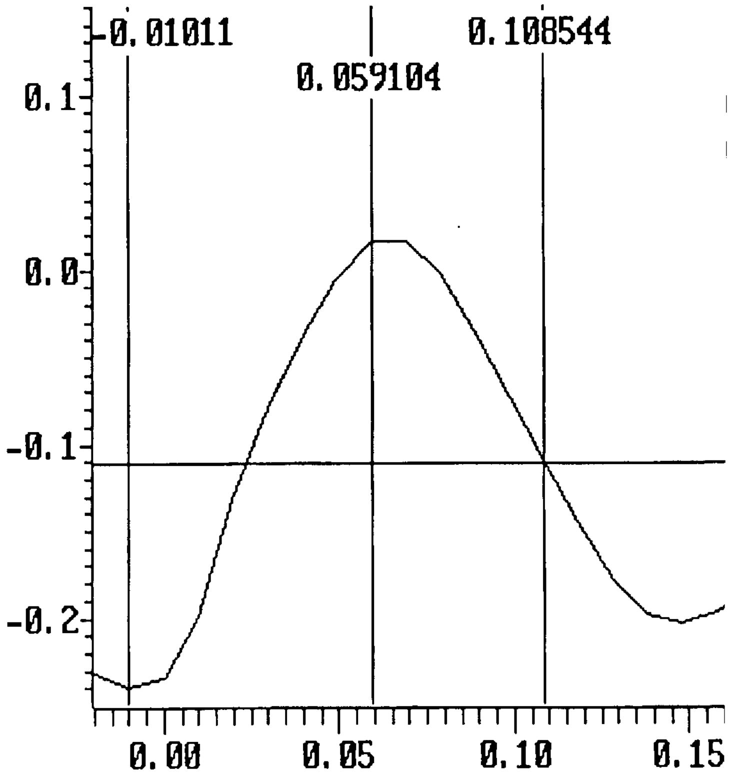


Figure 3.9 (a)

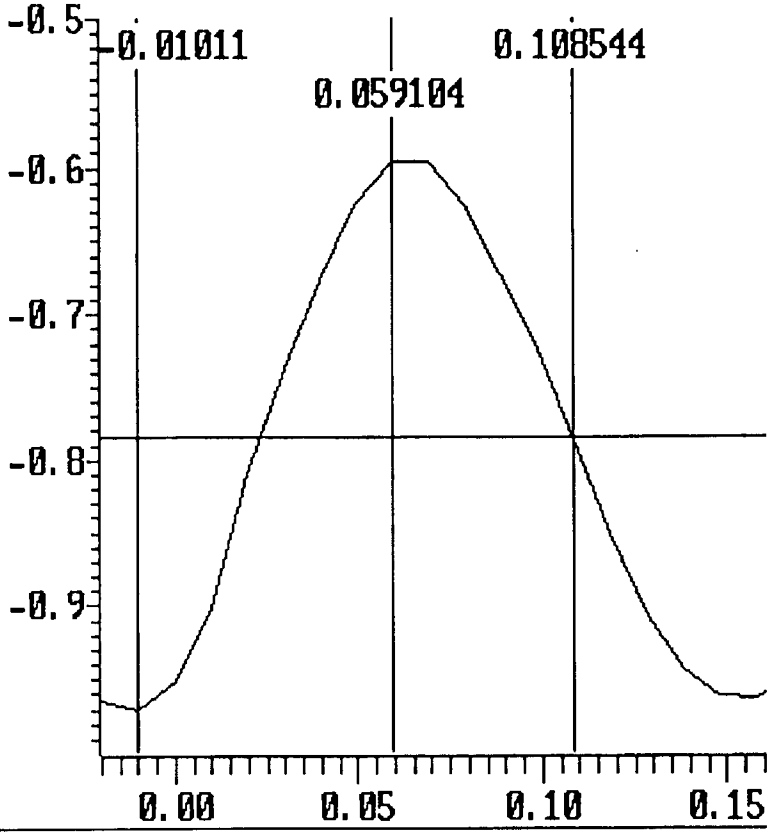


Figure 3.9 (b)

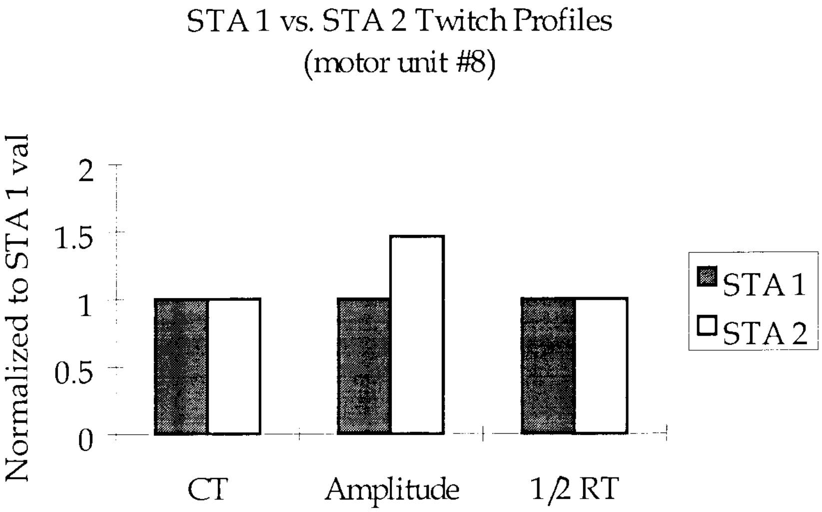


Figure 3.9 (c)

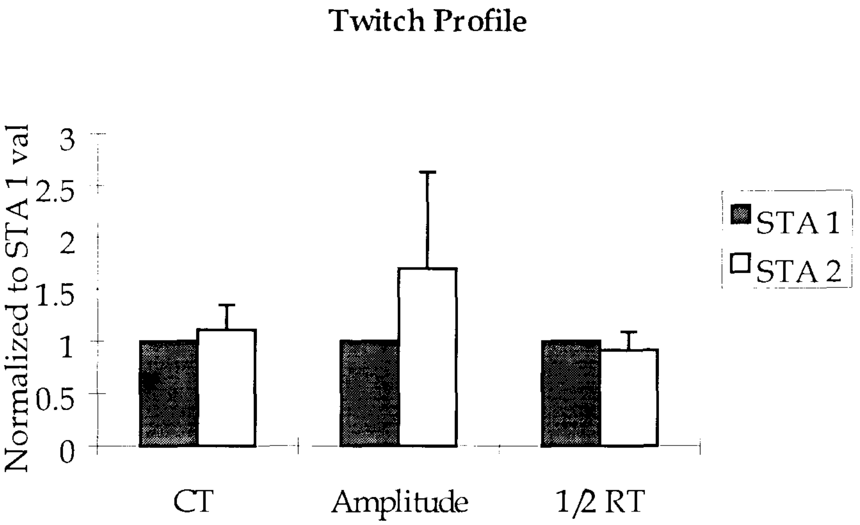


Figure 3.9 (d)

Figure 3.9. Twitch profiles for STA 1 versus STA 2. Changes are measured by the parameters contraction time (CT), Amplitude, and 1/2 relaxation time (1/2 RT). (a) Twitch profile of motor unit #8 measured during STA 1. Amplitude is 0.26 arbitrary units. (b) Twitch profile of motor unit #8 measured during STA 2. Amplitude is 0.38 arbitrary units. Note the difference in y-axis scale between (a) and (b). (c) A comparison of twitch profile parameters measured during STA 1 and STA 2 for motor unit #8. All values were normalized to STA 1 measured values. (d) A comparison of twitch profile parameters for n = 16 motor units. Error bars indicate standard deviation.

Twitch potentiation may have been caused by synchronous firing of motor units; therefore, all experiments were examined for synchronization of motor unit firing using rectified MUTA and the methods described in Chapter 2. Evidence of synchronization was not found in the sections of the experiments used for analysis.

No significant trends were found which distinguished the twitch profiles of high versus low threshold units in response to prolonged motor unit firing. Trends may emerge with the acquisition of additional data.

3.1.6 DC Force

DC force output of the first dorsal interosseous was monitored during constant rate recordings. Figure 3.10 shows the change in force output from the start to the end of the period of constant motor unit firing. No dependence was seen between the direction and/or magnitude of force change on the threshold of the motor unit tracked.

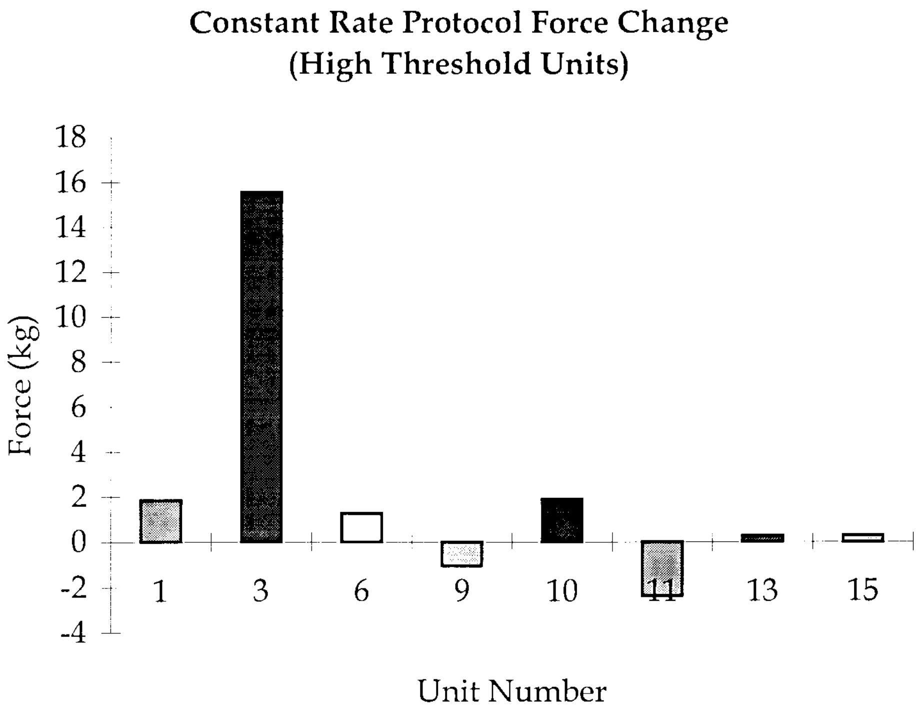


Figure 3.10 (a)

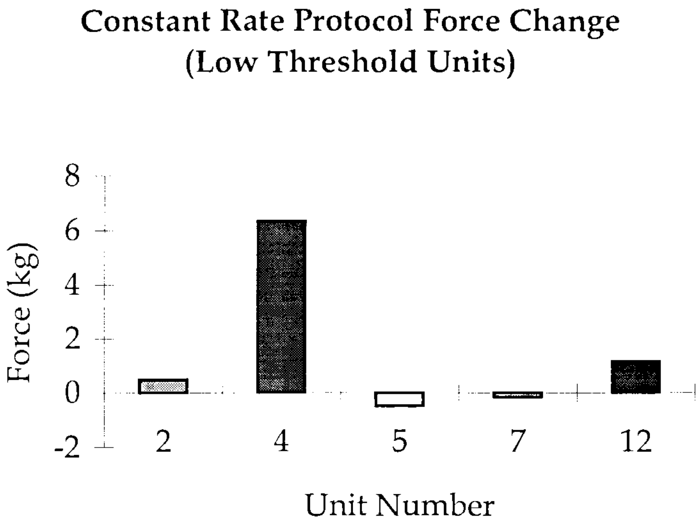


Figure 3.10 (b)

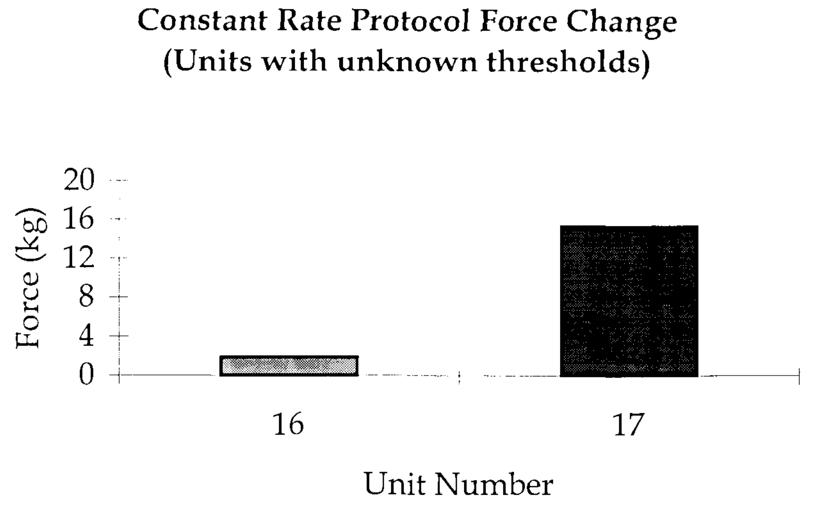


Figure 3.10 (c)

Figure 3.10. DC force change between the start and the end of the period of motor unit firing at a constant rate. (a) DC force monitored during the tracking of high threshold units. (b) DC force monitored during the tracking of low threshold units. (c) DC force monitored during the tracking of units with unknown thresholds. DC force was not obtained for motor unit #8.

3.2 Part II: Constant Force

3.2.1 Firing Rate of the Single Motor Unit

The second half of the experiment was the constant force paradigm (refer to Figure 3.2). As in Part I, motor unit firing rates were monitored. The mean firing rate of the 10 units ranged from 7.3 impulses per second to 13.2 impulses per second (mean ± standard deviation for all units = 9.1 ± 1.5 Hz) during the first two minutes of the constant force period (or one minute if the recording was less than four minutes). At the end of this firing period, mean rates ranged from 6.6 to 12.2 impulses per second (mean ± standard deviation for all units = 9.0 ± 1.8 Hz). In addition to the mean firing rate, the parameters mode firing rate and coefficient of variation were extracted from the first order interval histogram of inter-spike intervals (ISI). From start to end of the constant force portion of the experiment, motor unit firing rates were steady as measured by the mean and the mode firing rates (Figure 3.11). In addition, variability in motor unit firing did not increase as is indicated by a lack of change in the coefficient of variation. In fact, as illustrated by the example motor unit in Figure 3.11, firing variability of some units decreased by the end of the constant force period.

High and low threshold units were evaluated for differences in response to the constant force paradigm. None were found, but this may be due to the small sample size of each type of unit.

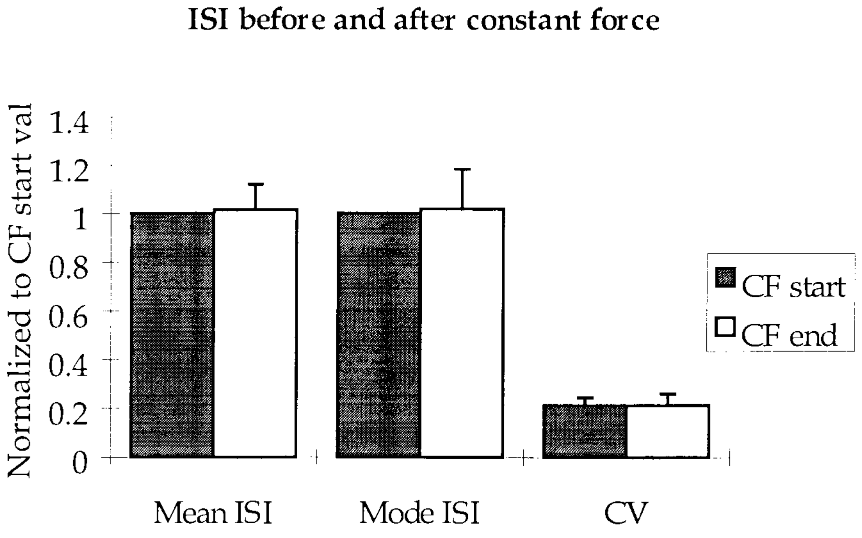


Figure 3.11 (a)

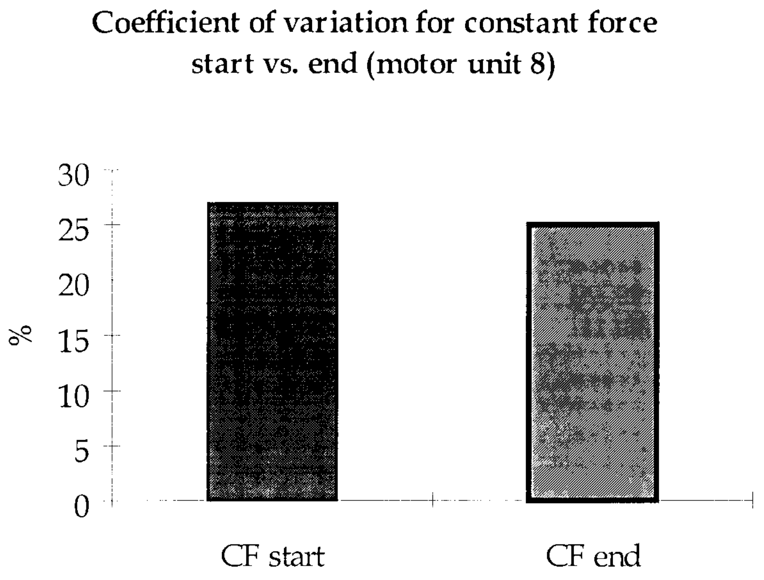


Figure 3.11 (b)

Figure 3.11. Effect of a constant force protocol on motor unit inter-spike intervals and variability of firing. (a) Inter-spike interval (ISI) mean, mode, and coefficient of variation at the start and end of maintained constant force for n=10 motor units. Mean and mode ISIs are normalized to start values. (b) Coefficient of variation of mean firing rate for motor unit #8.

3.2.2 EMG

As in Part I of the experiment, descending drive to the muscle's motoneuron pool was monitored in Part II using rectified surface EMG. Similarly, results show that from start to end of the constant force period, the magnitude of rectified surface EMG increased (Figure 3.12).

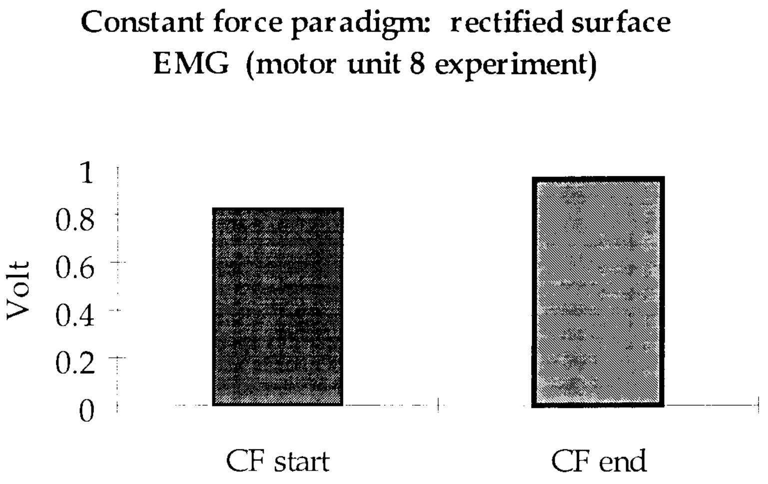


Figure 3.12 (a)

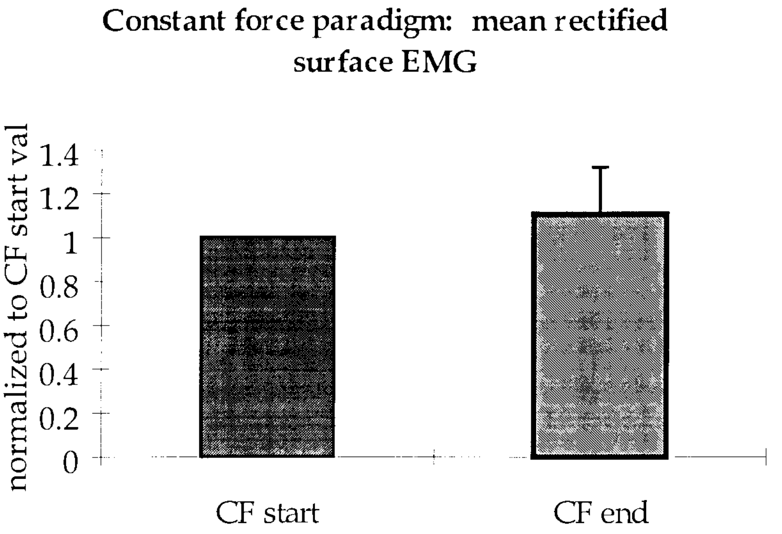


Figure 3.12 (b)

Figure 3.12. Rectified surface EMG at the start and end of a period of constant force (CF). (a) Raw values of rectified surface EMG for motor unit #8. (b) Rectified surface EMG at the start and end of constant force for n=10 motor units. Values were normalized to start values for comparative purposes.

3.2.3 MUTA

To monitor changes in muscle membrane properties, MUTA was measured during the constant force paradigm. The results show that unlike MUTA measured between STA 1 and STA 2 of the constant firing rate paradigm, the constant force paradigm did not result in a similar increase in MUTA's peak-to-peak amplitude from STA 3 to STA 4. Figure 3.13 illustrates the overall effect of the constant force paradigm on two parameters extracted from MUTA, peak-to-peak amplitude and peak-to-peak width, for the group of n=13 motor units. MUTA was measured during STA 3 and STA 4. The second parameter extracted from MUTA, peak-to-peak width, did not exhibit any clear trend in the direction of its change.

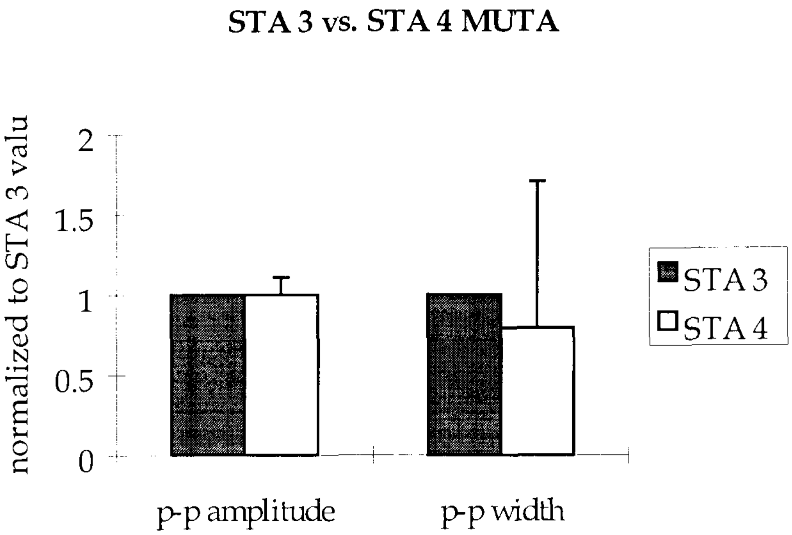


Figure 3.13. Effect of constant force on peak-to-peak amplitude and width for n=13 motor units. MUTA was measured during STA 3 and STA 4. To facilitate comparison, values were normalized to STA 3 measured values. Error bars indicate standard deviation.

MUTA was also measured at the start and end of the constant force period (Figure 3.14). Peak-to-peak amplitude did not change in a consistent direction for each of the motor units monitored: some motor units increased their amplitude while others decreased. This inconsistency is evident in the large error bars on figure 3.14(d). There was a significant increase (p=0.013) in peak-to-peak width of MUTA between start and end of the constant force period.

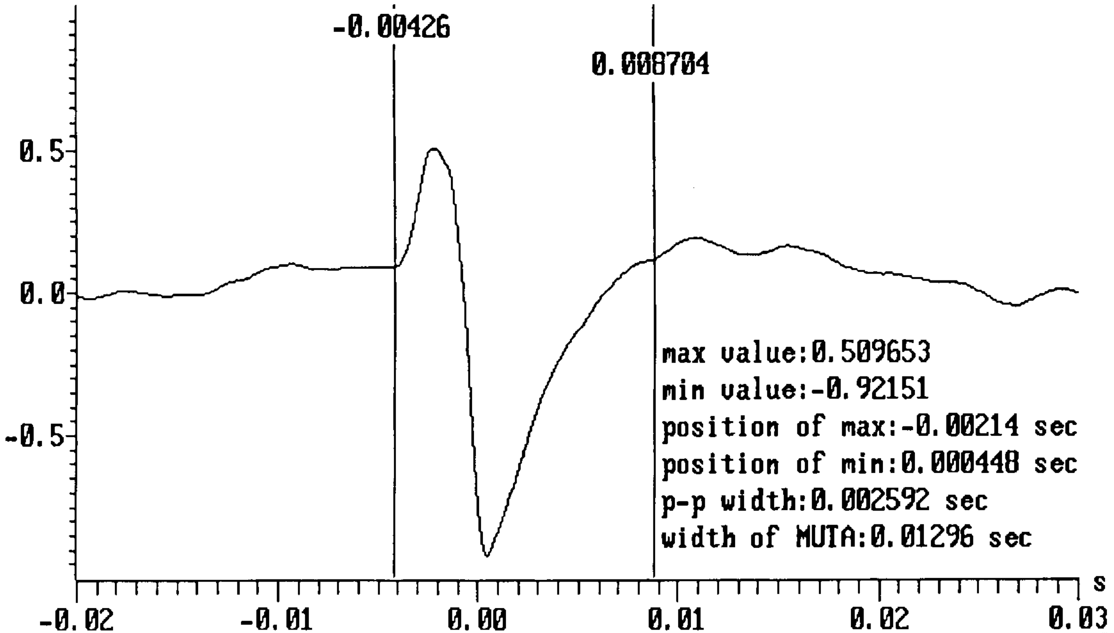


Figure 3.14 (a)

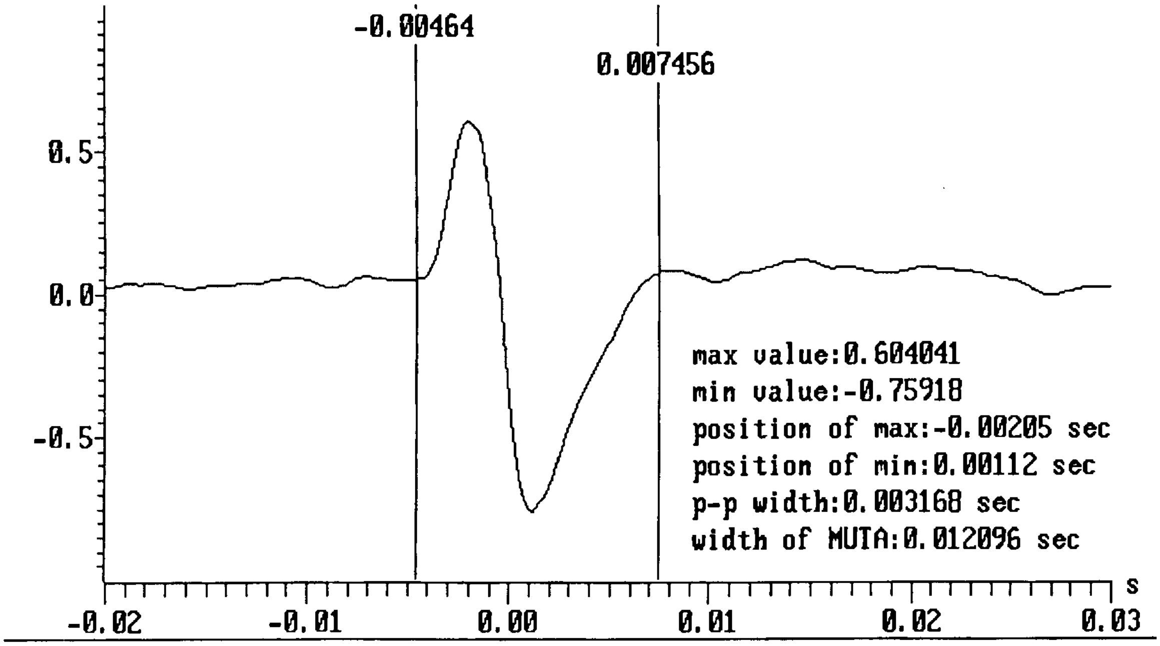


Figure 3.14 (b)

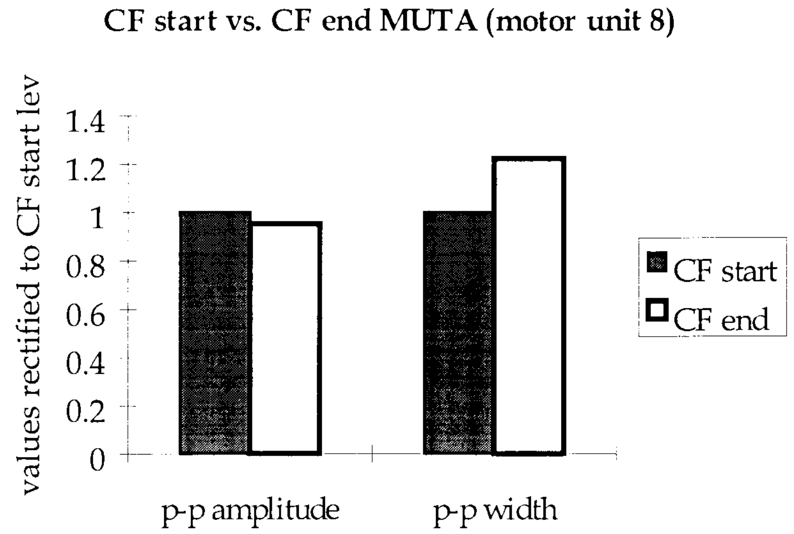


Figure 3.14 (c)

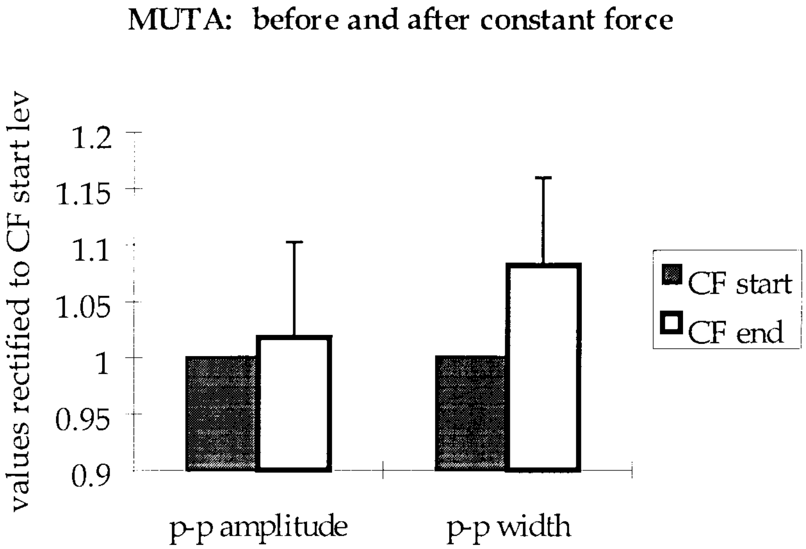
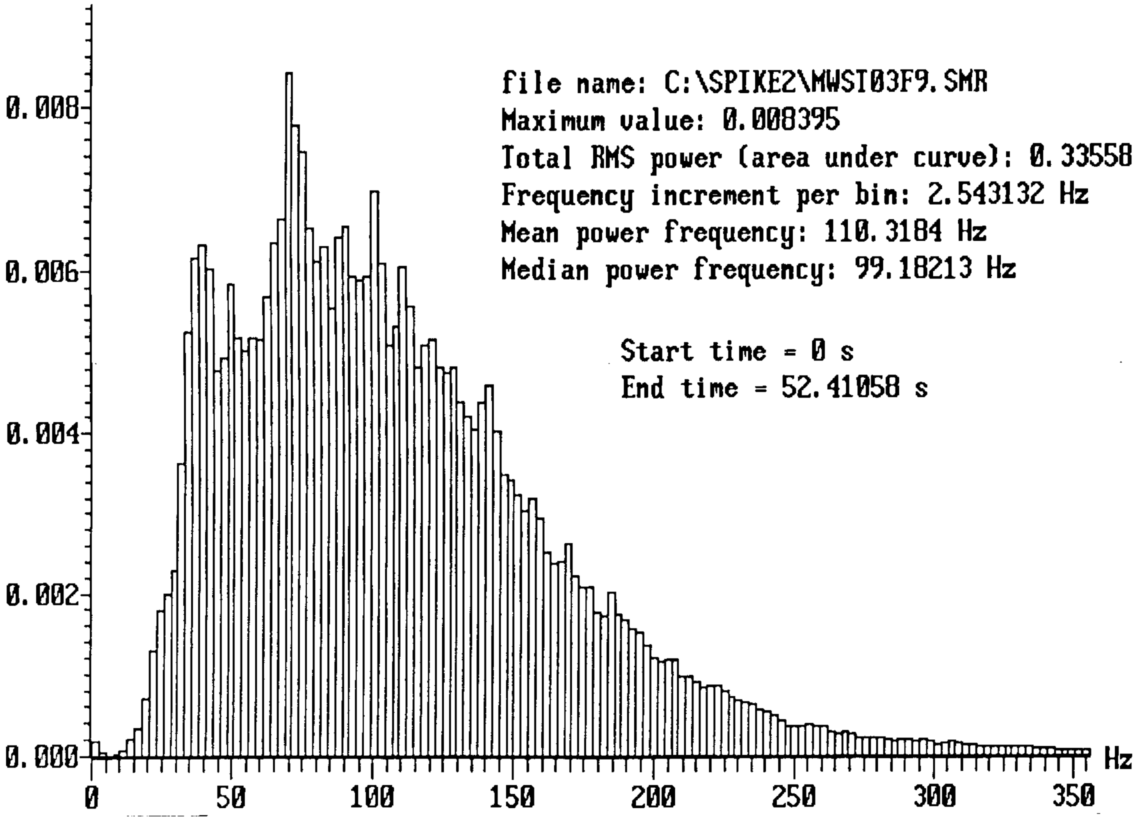


Figure 3.14 (d)

Figure 3.14. MUTA extracted during the start and the end of the constant force period. (a) Motor unit #8's MUTA measured during the start of constant force. (b) Motor unit #8's MUTA measured during the end of constant force. (c) A comparison of constant force start versus end peak-to-peak amplitude and width for motor unit #8. To aid comparison, values were normalized to start values. (d) This is the same comparison as shown in (c) but for n=10 motor units. Error bars indicate standard deviation.

3.2.4 EMG Frequency Spectrum

For the reasons given in section 3.1.4, the EMG frequency spectrum was monitored before (STA 3), at the start of, at the end of, and following (STA 4) the constant force protocol. The same parameters were extracted, RMS power and mean power frequency. Averaging over all experiments, RMS power showed a significant increase (p=0.03) before (STA 3) and after (STA 4) the constant force period (Figure 3.15). On the other hand, significance was not achieved (p=0.15) in the RMS power increase between the start and end of the constant force protocol (Figure 3.16). Although mean power frequency decreased between STA 3 and STA 4 and between the start and end of the constant force period, neither decrease attained significance (Figures 3.5 and 3.16).



```
file name: C:\SPIKE2\MWST03F9.SMR
Maximum value: 0.008395
Total RMS power (area under curve): 0.33558
Frequency increment per bin: 2.543132 Hz
Mean power frequency: 110.3184 Hz
Median power frequency: 99.18213 Hz

Start time = 0 s
End time = 52.41058 s
```

Figure 3.15 (a)

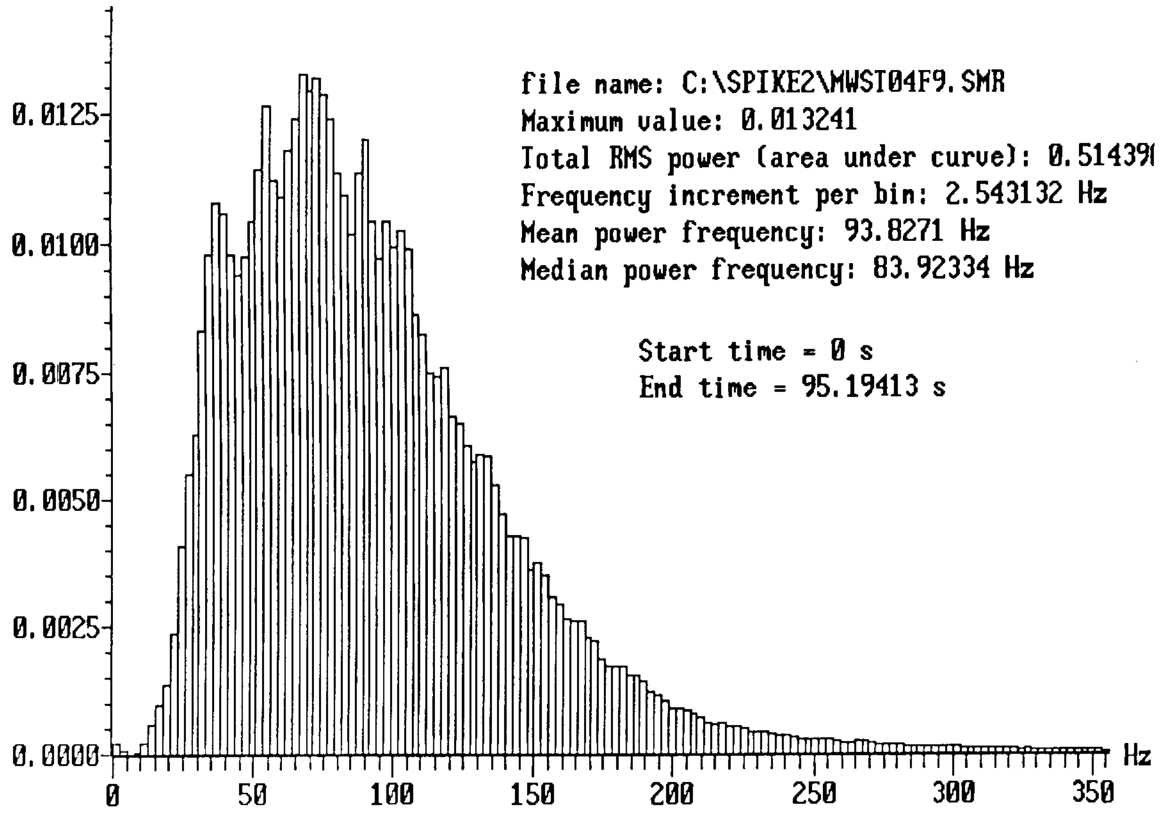


Figure 3.15 (b)

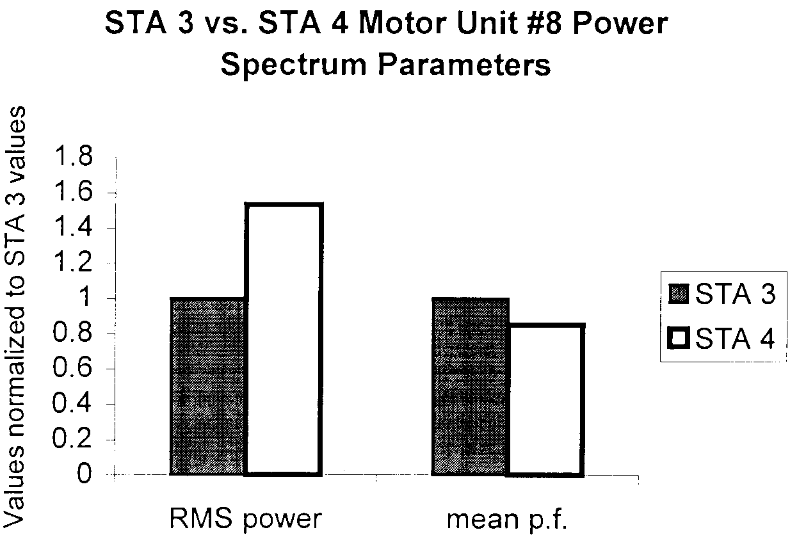


Figure 3.15 (c)

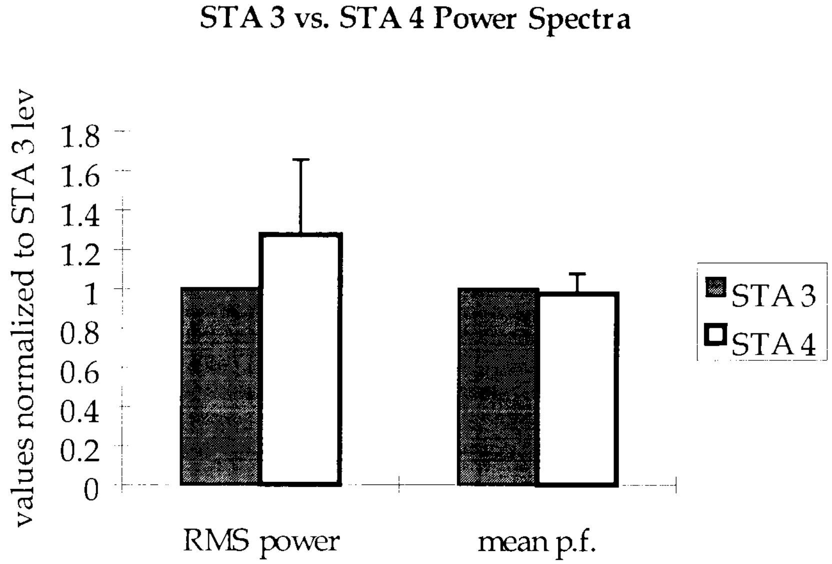


Figure 3.15 (d)

Figure 3.15. Surface EMG frequency (power) spectra for STA 3 and STA 4. (a) STA 3 frequency spectrum recorded when motor unit #8 was being tracked. (b) STA 4 power spectrum recorded when motor unit #8 was being tracked. (c) Comparison of power spectra parameters RMS power and mean power frequency recorded when motor unit #8 was being tracked. Values were normalized to STA 3 levels. (d) Overall view of change in RMS power and mean power frequency between STA 3 and STA 4 for n=10 motor units. For comparative purposes, values were normalized to STA 3 values. Error bars indicate standard deviation.

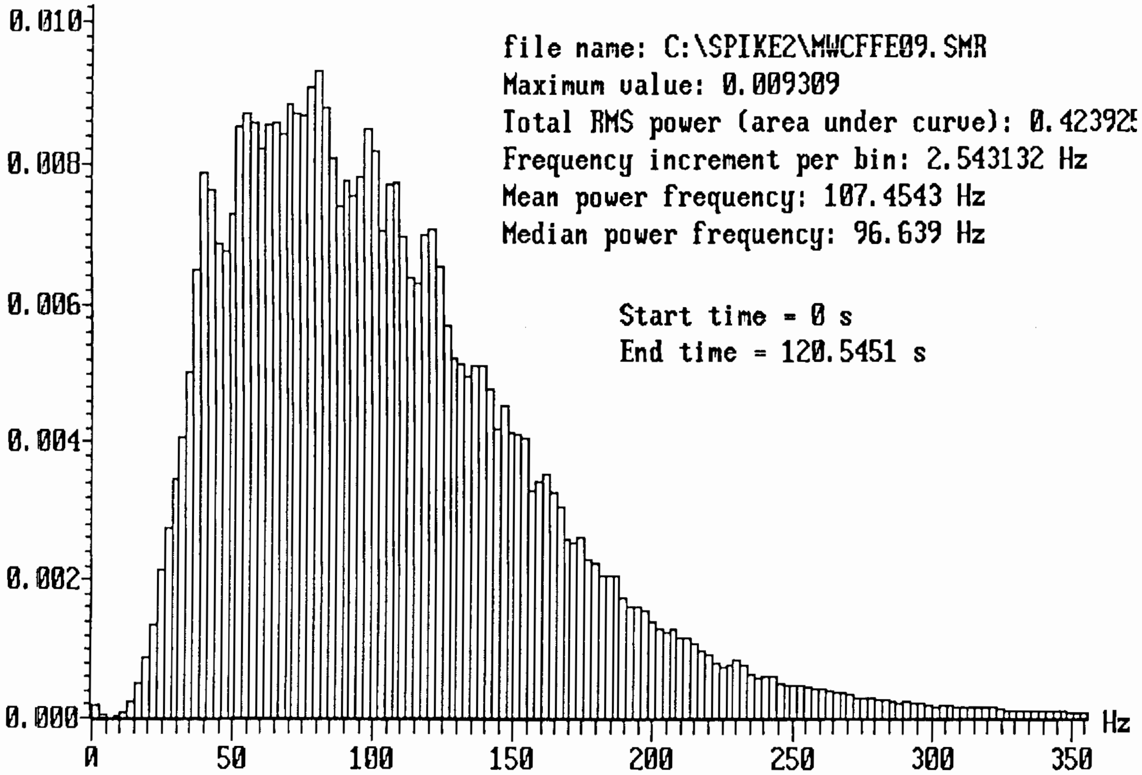


Figure 3.16 (a)

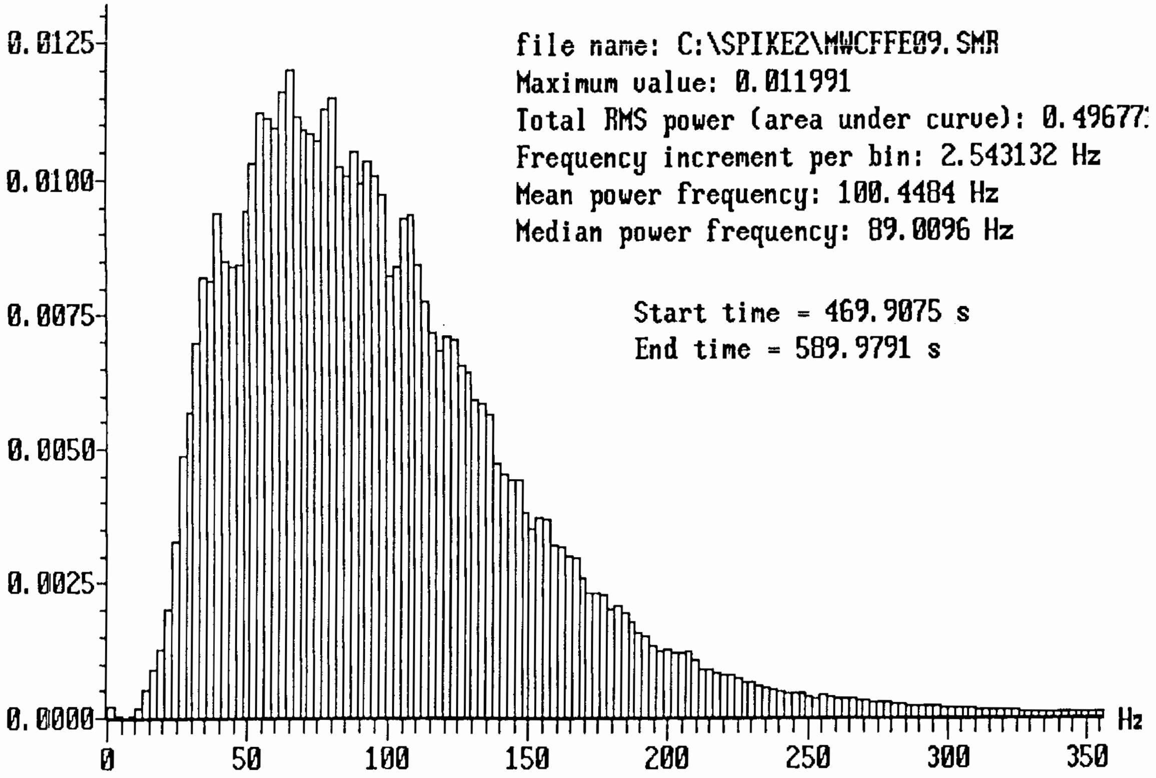


Figure 3.16 (b)

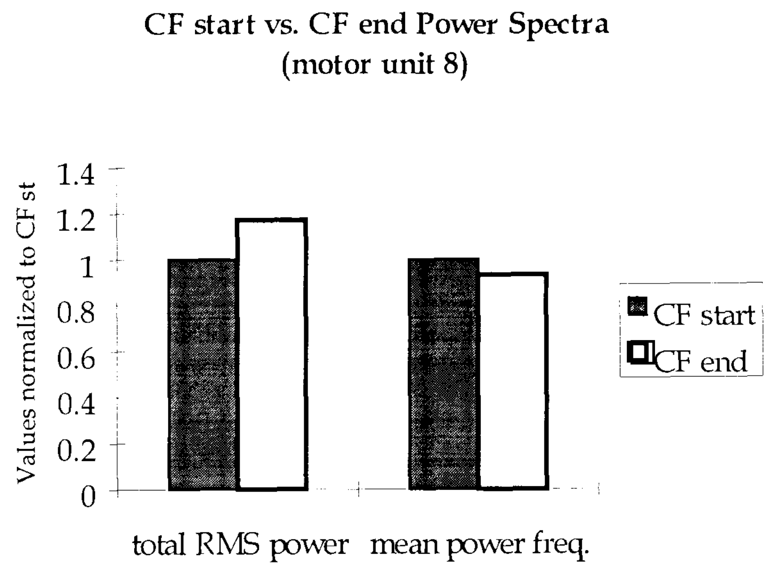


Figure 3.16 (c)

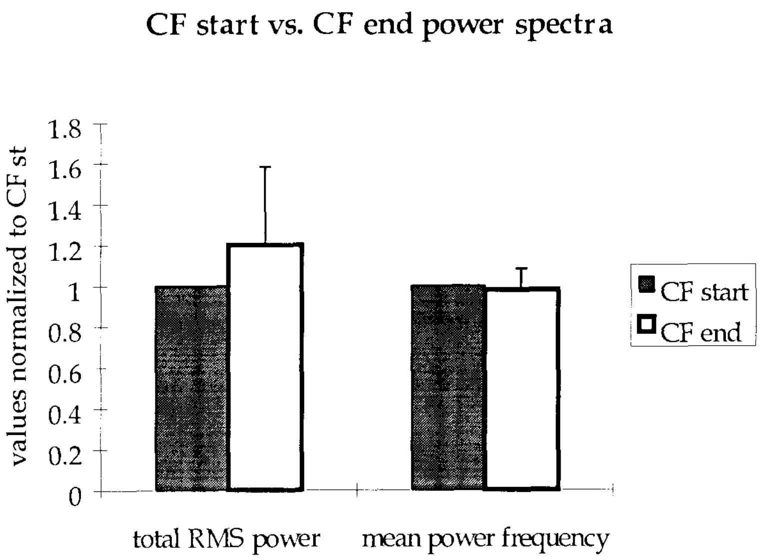


Figure 3.16 (d)

Figure 3.16. Power spectra measured at the beginning of a period of constant force versus that taken at the end. (a) Power spectrum measured at the start of the constant force period when motor unit #8 was being tracked. (b) Power spectrum measured at the end of the constant force period when motor unit was being tracked. (c) Comparison of start versus end total RMS power and mean power frequency recorded when motor unit #8 was being tracked. Values were normalized to start values. (d) Comparison of start versus end total RMS power and mean power frequency for n=10 subjects. Values were normalized to start values. Error bars indicate standard deviation.

3.2.5 Twitch Profile

As in Part I of the experiment, twitch profiles were extracted to obtain information relating to changes in motor unit contractile properties due to the prolonged holding of a constant force. Profiles were obtained using spike triggered averaging during the STA periods before and after the period of constant force. Three parameters were extracted from a twitch profile: contraction time (CT), amplitude, and 1/2 relaxation time (1/2 RT). No significant change in any of the twitch profile parameters was observed (Figure 3.17). Within the group of thirteen motor units, twitch amplitude increased for some units and decreased for others, but the overall effect was an increase which was statistically non-significant. The observed changes were not related to a unit's threshold classification (i.e., high or low threshold). Changes in contraction time and 1/2 RT were not significant.

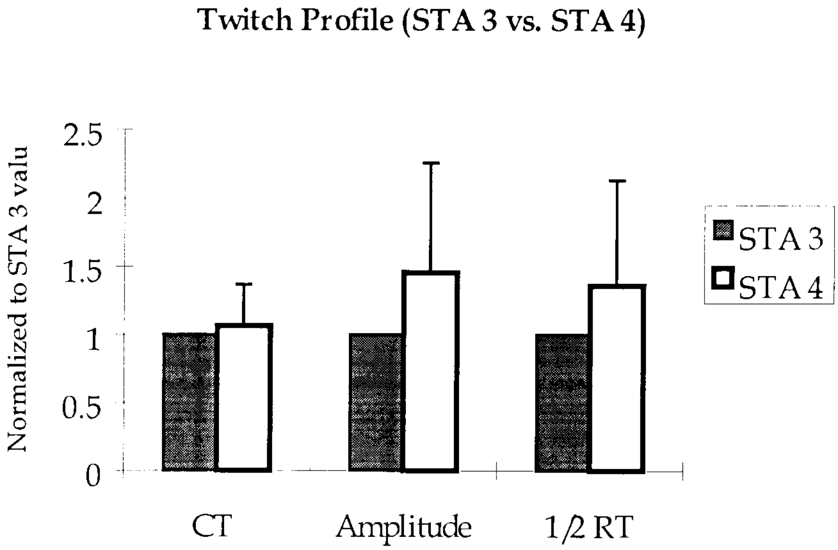


Figure 3.17. Twitch profile parameters compared before (STA 3) and after (STA 4) the period of constant force. The parameters, contraction time (CT), amplitude, and 1/2 relaxation time (1/2 RT), were measured for n=13 motor units Values were normalized to STA 3 values. Error bars indicate standard deviation.

A couple of subjects experienced muscle tremor for brief periods while they were holding a constant force. Muscle tremor could be accompanied by synchronization of motor unit firing and if it occurs, synchronization of motor units could account for an increase in twitch tension following prolonged motor unit activation. As a result, all experiments were checked for synchronization of motor unit firing using rectified MUTA and the methods described in Chapter 2. Evidence of synchronization was not found in the sections of the experiments used for analysis.

3.3 M-Wave

Supplementary M-wave experiments were performed to investigate fatigue induced changes in total muscle EMG. The aim was to correlate any alterations seen in the M-wave profile with those seen in MUTA. M-wave experiments were completed on three persons from the original pool of subjects. Stimulus pulses of 300 μs duration were applied to the ulnar nerve at the wrist. Intensity was increased until the M-wave, as recorded by surface electrodes on first dorsal interosseous (1DI), stopped increasing in size. Once maximum M-wave had been obtained, the subject was asked to fatigue 1DI by abducting the index finger for 10 minutes. Motor unit activity was not monitored during this time. Two experiments were maximum voluntary contractions (MVC) and two were at approximately 20% MVC.

M-wave profile changed dramatically following the fatiguing exercise. The examples of both MVC and 20% MVC contraction in Figure 3.18 show M-waves prior to and following the fatiguing exercise. Three parameters were measured on the M-wave: peak-to-peak amplitude, M-wave area, and peak-to-peak width. In the case of the MVC task, both peak-to-peak amplitude and M-wave area decreased while peak-to-peak width increased. This dramatic change in M-wave shape is especially evident in Figure 3.18 where the pre- and post-fatiguing exercise M-waves are superimposed.

The two experiments which used submaximal fatiguing exercise produced mixed results. In one experiment (Figure 3.18 b), peak-to-peak amplitude increased slightly, M-wave area decreased, and peak-to-peak width increased. The increase in peak-to-peak amplitude may have actually been due to changes in the shape of the M-wave. Prior to the fatiguing exercise the positive side of the M-wave had two peaks but following the fatiguing task there was only a single peak. After a few minutes of recovery, the M-wave regained its original shape where it had two peaks on the positive side. In the second experiment using submaximal fatiguing exercise, peak-to-peak amplitude decreased, M-wave area increased slightly, and peak-to-peak width increased. Due to the inconsistent results obtained following a submaximal fatiguing task, more data must to be collected before any conclusions can be drawn.

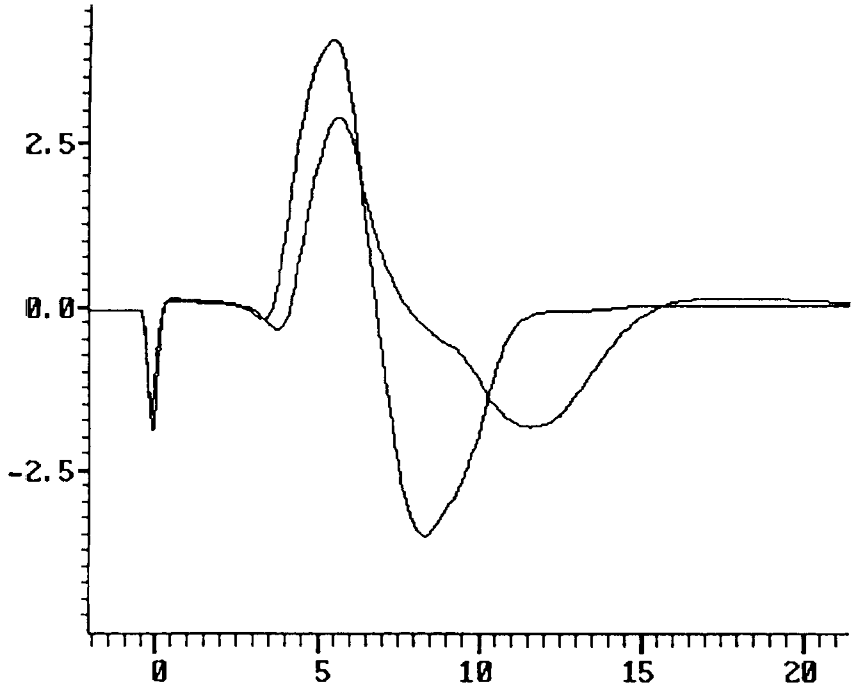


Figure 3.18 (a)

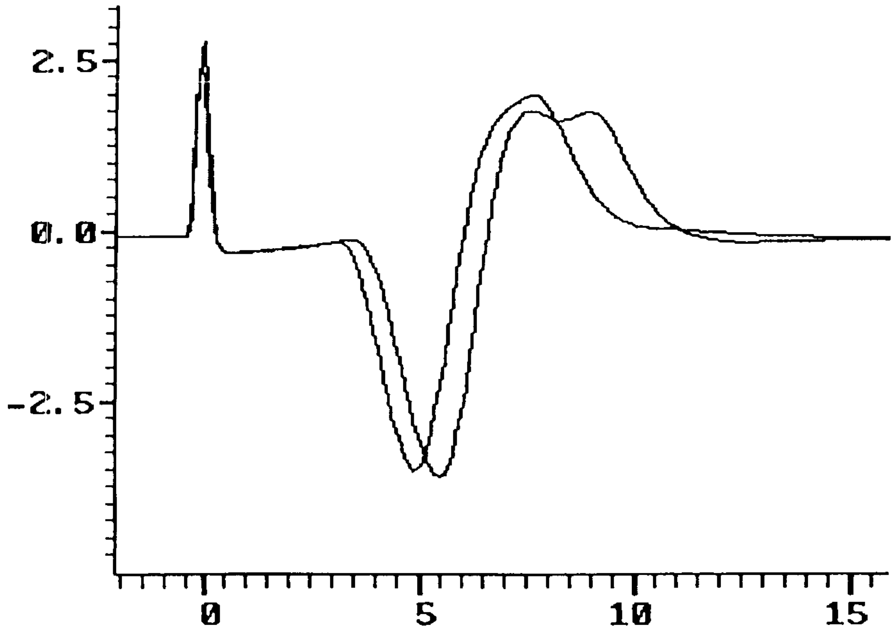


Figure 3.18 (b)

Figure 3.18. M-waves obtained prior to and following sustained isometric abduction of the first dorsal interosseous. The sharp spike in both figures is a stimulus artifact. The horizontal axis is time (ms) and the vertical axis is amplified voltage. (a) Pre- and post-MVC fatiguing contractions. The smaller M-wave is post-contraction. (b) Pre- and post-submaximal contractions. The M-wave with the single positive peak is post-contraction.

4.0 DISCUSSION

The objective of this study was to investigate the effect of prolonged motor unit activity on motor unit behaviour. The motor unit itself is one component of the neuromuscular control system and motor unit properties must be understood to gain further insight into human motor control. As described in Chapter 1, an increased understanding of human motor control is desired not only for medical applications but also among researchers in both biomedical and control systems engineering. Thus, to understand such a complex system, one focuses on a subsection of that system. In this case, the system was neuromuscular control and the subsystem was the motor unit, the final common path for all motor commands.

Human and animal motor units have been well studied under a variety of different conditions. In this work we focused on the response of the unit during conditions of prolonged activation. Such an approach has rarely been used with human subjects, particularly for periods as long as ten minutes (perhaps because tracking a motor unit for ten minutes is an especially challenging task). When considering prolonged motor unit activation, results from fatigue studies may be relevant and have therefore been considered.

A variety of aspects of the motor unit were focused upon in this study: the motoneuron and its firing rates, muscle membrane properties, and motor unit twitch profiles. In addition, central or descending drive to the motor unit (and muscle) was assessed.

4.1 Motoneuron Firing Properties

In the current study, inter-spike intervals (ISI) of the motor unit were tracked during prolonged periods of (a) a constant firing rate and (b) a constant force. With prolonged activity, variability in inter-spike intervals was expected to increase, as had been reported for experiments using force-defined protocols (Gatev et al., 1986; Enoka et al., 1989). In an unfatigued motoneuron, variability in ISI depends on the firing rate, the higher the firing rate, the lower the variability (Jones and Bawa, 1997); therefore, in this study variability was examined at a constant firing rate. In the present study, mean ISI variability for the group of ten motor units, as measured by the coefficient of variation, was 0.20 at the start and end of the period of prolonged firing . However, since there was a statistically significant increase in firing rate (mean firing rate increased from 9.8 ±1.6 Hz to 11.0 ±2.2Hz for the group, p=0.01) during the constant firing rate paradigm, one cannot conclude that the coefficient of variation would not change following a period of prolonged firing at a constant rate. With this in mind, the method of monitoring mean ISI should be improved for future experiments so that the method allows the subject to more accurately monitor their firing rate but which does not result in overcompensation (i.e., time averaging and smoothing required). Suppose we had been able to maintain a constant firing rate, would the coefficient of variation have increased? Although the natural tendency in this study was to increase firing rate with prolonged activity, the results of the constant force paradigm, as described below, suggest that the coefficient would not increase.

The constant force paradigm was used in the current set of experiments to facilitate direct comparison to both the constant firing rate portion of the experiment as well as experiments by other researchers. As in the constant rate section, no increase in coefficient of variation was observed in the constant force portion. The coefficient of variation remained at 0.21 at the start and end of the period of constant force. For the group of motor units, firing rate decreased slightly from 9.08 ±1.55Hz to 8.95 ±1.83Hz which was not significant (p=0.45). This decrease in firing rate is similar to fatigue paradigms in which a decrease in motoneuron discharge has previously been demonstrated (Bigland-Ritchie et al., 1983); however, little value should be put on the change in the present experiment because it was not significant. Still, individual motor units showed a variety of changes in ISI following the constant force activity: some units had increased ISIs, some stayed the same, and others decreased.

Contrary to the results of the present experiment, Gatev et al. (1986) reported a fatigue-induced increase in variability of motor unit firing. Their experiment was designed to measure changes in the coefficient of variation while maintaining a constant force output. The group used what were assumed to be flexor pollicis brevis motor units recruited at 40 to 80% MVC. Surface electrodes, rather than invasive needle electrodes, recorded motor unit activity and consequently the researchers were not entirely sure of the muscle from which the motor units were being recorded (they may have picked up units from opponens pollicis and adductor pollicis). The contraction used in the study was a sustained apposition of the thumb to the index and middle fingers. Gatev's group reported an increase in the coefficient of variation of the ISI during the time between recruitment and refusal to fire. Coefficient of variation (CV) was measured in the first ten seconds of the spike train where values ranged from 0.10 to 0.59 (mean ISI of 31.3 ±11ms) and during the last ten seconds where values ranged from 0.18 to 0.80 (mean ISI of 75 ms). These increases in CV were accompanied by concomitant changes in firing rate; therefore, a conclusion cannot be drawn as to whether the increases in CV were due to fatigue or increases in ISI.

In a second study, an increase in CV following fatiguing activity was reported (Enoka et al., 1989). This experiment used the first dorsal interosseous (1DI) muscle in an abduction task; however, rather than a sustained contraction, a cyclic ramp and hold protocol was employed to induce rapid fatigue. The objective of this portion of their study was to see how interspike-interval variability would be affected by a fatiguing task. Enoka's group used a subjectively determined endurance limit (i.e., when the subject could not meet the 50% MVC target force) whereas the present study used a pre-determined duration. For 10 of 17 motor units, an increase in CV was observed following the fatiguing ramp and hold trials.

Contrary results were obtained by Miller et al. (1996) who observed no increase in ISI variability with increasing fatigue. This study used biceps brachii motor units (recruitment thresholds of 4.2 to 54.4%) in a repeated elbow flexion and extension contraction task lasting 8.3 minutes. The task included a sustained isometric component. As was also noted by Enoka et al. for their experiment, Miller's group did not observe a uniform change in motor unit inter-spike intervals due to fatigue: some increased, some decreased, and others stayed the same. In the constant force paradigm of the current study, at the end of the period of constant force two of ten units had decreased ISIs, four of ten units had increased ISIs, while the rest were approximately the same.

We also examined changes in the coefficient of variation prior to and following the constant rate and constant force periods (during spike triggered averaging). During these times, subjects were given the difficult task of firing the tracked motor unit at a slow rate (where the mean ISI > 100 ms). Before the constant rate period (STA 1) the average CV was 0.22 while after the constant rate period (STA 2) the average CV was 0.25. This was not a significant increase as measured by the student's t-test (p=0.10). As well, the firing rate did not change significantly between these periods (p=0.18). Before the constant force period (STA 3) CV = 0.21 and after the constant force period (STA 4) CV = 0.24. Unlike the change in CV before and after constant firing, this increase in variability was significant (p=0.03) and the mean firing rate did not change significantly (p=0.31).

Actual CV values were provided in the aforementioned studies and ought to be compared with the current study, particularly those studies where 1DI was used. In the Enoka study, the mean coefficient of variation (measured while target force was maintained) was 0.29 ±0.09 for the abduction task (mean ISI of 87 ± 17ms). Coefficients of variation for all units studied ranged from 0.15 to 0.49. In the current study, coefficient of variation values were between 0.20 and 0.25 for ISIs which ranged from 76.0 to 151.3ms. Because the mean firing rates of the Enoka and the current study were different and given that the coefficient of variation (CV) is directly proportional to the inter-spike interval (Jones and Bawa, 1997), actual CV values cannot be compared. Furthermore, motor units used in the Enoka study were restricted to those recruited at forces up to 15% MVC. Although the trend in the Enoka study was towards an increase in the coefficient of variation, a range of pre and post fatigue firing behaviours were observed: the majority of units had increased ISIs, some had unchanged ISIs, while others had decreased ISIs. In another study measuring the coefficient of variation of 1DI motor unit firing, Freund et al. (1972) reported coefficients in the range of 0.12 - 0.25 with firing rates of between 5 and 20 Hz. In this work, subjects were required to maintain an isometric abduction for only 0.5, 1, or 2 minutes.

The current experiments have shown evidence that variability of motor unit firing does not increase when units fire at comfortable rates for prolonged periods. To date, different studies have either shown an increase (Gatev et al., 1986; Enoka et al., 1989) or no change (Miller et al., 1996) in coefficient of variation with prolonged firing. In constant force experiments, increases in CV are accompanied by increases in ISI; therefore, it is difficult to say whether CV would have increased without a change in ISI. In the constant force paradigm of our study, no increases in ISI or CV were observed. The current study (constant rate portion) is the only one where an attempt was made to keep ISI constant and no changes in CV were observed (although this portion of the study needs to be repeated with care since it was unsuccessful). Consider also that quantitative comparisons of CVs are difficult because the same firing rates in two different pools may have completely different inherent coefficients of variation, or the motoneurons tracked may have been of different types. For example, a fast motoneuron firing at 10 impulses/second has a higher CV than a slow, soleus motoneuron (Jones and Bawa, 1997). As described in Chapter 1, an increase in variability of firing is due to noise at the motoneuron's cell body and proximal dendrites. Noise impacts a slower firing motoneuron more than a faster firing neuron due to differences in shape of their AHP (after-hyperpolarization) trajectories (Jones, 1995; Matthews, 1996). Consequently, evaluations of changes in the coefficients of variation should be done when the motor unit is firing at a slow or moderate rate.

4.2 Central Drive

In these experiments, central drive to the muscle was monitored using two methods: (1) full-wave rectified average surface EMG and (2) total power of the entire unrectified EMG signal, expressed as root mean square power (RMS power). Researchers have observed that during constant force protocols at submaximal levels, EMG amplitude and total (RMS) power of the power spectrum increase (Petrofsky et al., 1982; Moritani et al., 1982; Krogh-Lund and Jørgensen, 1991; Cresswell et al., 1996). As expected for the current experiments, when force was held constant, central drive increased from start to end of the period of constant force. The constant rate section of the experiment also demonstrated an increase in rectified EMG and RMS power. An increase in surface EMG amplitude and RMS power of the unrectified EMG signal may be attributed to recruitment of additional motor units. Indeed, recruitment of new motor units was observed during the constant rate and constant force protocols.

During constant force protocols of the current experiment, mean rectified EMG increased while motor unit inter-spike intervals increased (without attaining significance). Consequently, the observed increase in descending drive to motor units during a constant rate protocol was expected. However, explanations for what was expected and what was observed when comparing the two protocols disintegrate at this point. In the current set of experiments, the constant force protocol behaved as expected: force was constant and mean firing rate decreased slightly as rectified EMG increased slightly. As a result of increased central drive to the pool, additional motor units were seen, as picked up by the intramuscular recording, but the activity of these additional single units was not quantified. The constant rate protocol, on the other hand, actually showed an increase in mean firing rate concurrent with an increase in rectified surface EMG. This was expected; however, as rectified surface EMG increased and firing rate increased, force output of the muscle remained constant. Given what was seen in the constant force protocol, an increase in force output was expected for the constant rate protocol. Recall that the magnitude of force during the constant force protocol was set at the level at which the unit being tracked was firing at the same rate as it was during the constant rate protocol. In the end, the forces maintained during both the constant rate and constant force parts of the experiment were similar. Additional motor units were also recruited during the constant rate protocol, but as in the constant force protocol, neither their numbers, nor the time they were recruited, was considered. One study in which recruited units were considered was performed by Nordstrom and Miles (1991) who investigated the activity of surrounding motor units while focusing upon the activity of a single unit. In a study using the masseter muscle, they required subjects to maintain the mean firing rate of a single motor unit at 10 Hz for 15 minutes. While doing so, they studied the activity of one to four motor units which were or became active while the unit was firing and found a statistically significant ($p<0.01$) change in mean firing rate in 58% of the units. Their results may provide an explanation of how the mean firing rate of a unit and the rectified EMG could increase while force output remains constant: the size-related order of motor units' firing rates and activity can be selectively changed during periods of prolonged firing.

An increase in descending drive, required to maintain a constant force output, may cause an increase in firing rate of already recruited units in addition to recruitment of new units. This is because the motor unit under study is recruited at a certain level of descending drive. Over time, the unit has greater difficulty firing at a regular rate and therefore requires an increased level of drive to keep firing. With this increasing drive, additional units are activated and the unit sometimes fires at a faster rate. Conversely, when the unit has not been active for a prolonged period, an increase in firing rate and EMG magnitude produces an increased force output of the muscle.

To summarize, the current experiments have shown that motor units experience changes in their properties due to prolonged activation at submaximal levels. In the experiments, an increase in descending drive was reflected by a corresponding increase in motor unit firing rates but despite the increased drive to the muscle and the greater firing rate of the unit studied, the force output of the muscle did not increase. This suggests that during prolonged activation, the contractile machinery of the muscle fibre or the excitation-contraction mechanism are what is first affected by prolonged motor unit firing.

Also worth considering is how the desired task affects descending drive to a muscle task group. Compare the two different control problems faced by the CNS in the current experiment: during the first half half, subjects were required to maintain a constant firing rate whereas in the second half, a constant force output of the muscle was required. Force output was similar for both tasks, but only the constant rate protocol showed a significant increase in firing rate and rectified EMG. Also consider the following as an illustration of the role of task effects in this experiment: although the force maintained in the two tasks at hand was similar, subjects could fire the motor unit for a longer duration for the constant rate protocol than they could for the constant force protocol. Why was this the case? And how does the CNS approach the two problems? Finally, while on the topic of task effects, differences have been observed in changes in muscle fibre contractile properties for submaximal versus maximal voluntary contractions (Gandevia, 1992; Bigland-Ritchie, 1993).

4.3 Muscle Membrane Properties of the Motor Unit

MUTA was used to monitor changes in the muscle membrane properties of the unit being tracked. MUTA is typically regarded as the signature of a motor unit (Milner-Brown and Stein, 1975); however, in these experiments changes were observed in the shape of MUTA as a result of prolonged firing.

Comparing MUTA before and after the period of constant firing, MUTA's amplitude increased significantly. As well, a slight increase was seen in the peak-to-peak width of MUTA. Such changes were not seen between the start and the end segments of the period of constant firing. An increase in MUTA amplitude is a result of greater depolarization of the muscle fibre membrane. Depolarization of the membrane is caused by Na^+ moving into the cell through voltage-gated channels and this was potentiated in the period following constant firing of the unit. Neuromuscular junction transmission does not appear to fail during or following prolonged motoneuron firing.

The reason for this increase in MUTA's amplitude following the period of constant firing is not understood. The period following constant firing is when the motoneuron firing rate has decreased to below 10 impulses per second and the force output requirements of the muscle have also decreased. At this time, the evidence points to the probability of an increase in ACh release from the motoneuron terminal, causing a greater end-plate-potential (EPP), thereby causing the opening of a greater number than usual voltage-gated Na^+ channels.

Why this occurs is not understood. To test for this mechanism, a neurotransmitter blocker such as edrophonium, an anticholinesterase, would have to be used. Broadening of the peak-to-peak width is probably due to a slowing of the conduction velocity of the muscle fibre action potential across the sarcolemma. As described in Section 1.3.4, this could be due to altered conductances of the various ions (Cl^-, Na^+, K^+) or to K^+ ion accumulation in the t-tubules (Edman, 1995).

No changes were observed in MUTA between STA 3 and STA 4 but this could be attributed to the shorter duration of many of these trials (four minutes) in the constant force protocol versus ten minutes for the constant rate protocol.

For the constant force protocol, inconsistent changes in the MUTA amplitude were observed from start to end: some units increased in amplitude and some decreased. The changes could not be attributed to motor unit threshold and could not obviously be attributed to any other factors. At the same time, peak-to-peak width of MUTA increased.

4.4 EMG Frequency Spectrum

Changes observed in the EMG frequency spectrum were similar for both the constant firing rate and the constant force protocols. The frequency at which the mean power in the EMG signal occured (mean power frequency) shifted to a lower value (although not significantly) when compared before and after the constant firing period, before and after the constant force period, and between the start and end segments of the constant rate and constant force protocols. This result has been observed by many researchers in fatigue studies (Komi and Tesch, 1979; Moritani et al., 1982; for review see De Luca, 1984; Fuglevand et al., 1989; Krogh-Lund and Jørgensen, 1992; Fuglevand et al., 1993).

Komi and Tesch (1979) had subjects perform repeated MVCs of the m. vastus lateralis muscle (knee extensions) and measured the degree of fatigue in the muscle by a decline in force. With increasing fatigue, they observed a significant decrease ($p<0.01$) in mean power frequency of the surface EMG signal in individuals with a high percentage of fast-twitch muscle fibres (a muscle biopsy was taken). Moritani et al. (1982) used the biceps brachii and soleus muscles in their experiments. Subjects performed sustained MVCs and submaximal contractions for as long as possible. Along with observing a decline in the mean power frequency with fatigue, Moritani et al. observed that those motor units with a higher initial mean power frequency (measured during MVC) would fatigue more than those with a relatively lower mean power frequency (i.e., show a greater drop in frequency). In 1989, Fuglevand et al. modelled, using a motor unit pool model, the effect of an increased motor unit action potential duration on the amplitude and mean power frequency of surface EMG. With increased motor unit action potential duration, full-wave rectified average EMG increased and mean power frequency decreased. Krogh-Lund and Jørgensen (1992) examined isometric flexion of the elbow at 15% of MVC and recorded surface EMG from biceps brachii and brachioradialis muscles. They used the median rather than mean power frequency (refer to Section 2.5.6) and reported a significant drop from initial values (~66%) with prolonged flexion. More recently, Fuglevand et al. (1993) reported a drop in 1DI surface EMG mean power frequency. Their protocol was similar to the constant force portion of the current study: subjects performed isometric abduction of the index finger at 20, 35, and 60% of MVC until the force dropped below the target level.

The following is a list of factors which could cause a downward shift in mean power frequency of the EMG: a decreasing muscle fibre conduction velocity, motor unit synchronization, recruitment of new motor units, and variability of motor unit firing rate. Note that an increase in the variability of motor unit firing would preclude an increase in motor unit synchronization. In the current study, motor unit synchronization was tested for using rectified MUTA and was not found. Firing rate variability did not increase during either the constant firing rate or the constant force protocols.. Unfortunately, recruitment of new motor units was only observed and not quantified in these experiments; nevertheless, recruitment was not believed to be the most significant factor causing the decrease in the frequency at which the mean power of the EMG signal occured. Recording was halted before the number of units firing in the vicinity of the needle electrode became too great due to increasing difficulty in tracking the motor unit. Therefore, the factor that is believed to play the greatest role in decreasing the mean power frequency is a decrease in conduction velocity of the action potential across the muscle fibre. This conclusion is also supported by the observation that peak-to-peak width of MUTA increased.

4.5 Twitch Profile (Contractile Properties)

The force generating system of the motor unit was examined using twitch profiles. A significant increase in twitch amplitude was seen between periods recorded before (STA 1) and after (STA 2) prolonged constant firing. Similar increases in twitch amplitude have been reported in experiments using a submaximal force protocol. This increase in twitch amplitude is termed postactivation potentiation (Burke, 1981). Similarly, twitch amplitude increased between periods recorded before (STA 3) and after (STA 4) constant force although the increase did not attain significance (p=0.07). this could have been due to the small sample size and the duration of time for which a constant force could be held by the majority of subjects. Subjects had greater difficulty holding a constant force for ten minutes than they did holding a motor unit firing at a constant rate.

As described in Section 1.3.5, potentiation of twitch amplitude may be due to changes in calcium kinetics such as slowed re-uptake of calcium during excitation-contraction coupling (Burke, 1981). A second possible mechanism causing potentiation is surplus calcium release (Edman, 1995). One would expect a decline in twitch amplitude with prolonged usage, but as was demonstrated by Edman, an increase in twitch force of an individual unit does not translate into a corresponding increase in force output of the muscle. As described in Section 1.3.5, potentiation of twitch force does not translate into an increase of the tetanic force of the motor unit and since the tetanic force has not increased, the force output of the muscle does not increase. An interesting future study would involve examination of the time course of twitch potentiation following prolonged activation of a motor unit. The time course of recovery of twitch magnitude could be compared with fatigue recovery, as defined by MVC or M-wave recovery.

Between the periods recorded before (STA 1) and after (STA 2) the period of constant firing, contraction time increased (p = 0.09) and 1/2 relaxation time decreased (p = 0.08). These changes, although not significant for this sample size, reflect changes in the kinetics of the myosin cross bridges and calcium re-uptake by the sarcoplasmic reticulum (Viitasalo and Komi, 1981). For the constant force protocol, contraction time and 1/2 relaxation time changes were inconsistent. This may be due to the length of time that subjects were able to hold a constant force before losing the unit being tracked or before it became too difficult to track the unit. The constant force was held for shorter periods than subjects were able to hold the constant firing rate, which may have contributed to unstable or transient effects.

4.6 M-wave

Although the M-wave tests were preliminary trials, their results are relevant to changes seen in the shape of MUTA in these experiments. The M-waves were performed to examine whether the observed increases in MUTA's amplitude following prolonged motor unit activation would be reflected in a corresponding increase in M-wave amplitude. Despite their limitations, results of the M-wave experiments provide some support to the changes seen in MUTA during the experiments. In both MVC and submaximal M-wave experiments, peak-to-peak width of the M-wave increased, supporting the notion of a slowing conduction velocity across the sarcolemma as a result of prolonged activity. One M-wave increased in amplitude following ten minutes of submaximal contraction. The other experiment using submaximal contraction resulted in a decrease in M-wave amplitude. The inconsistency of results parallels what was observed for MUTA during the constant force period. Fuglevand et al. (1993) performed a similar yet more comprehensive study of the effect of submaximal exercise on M-wave amplitudes. They found that M-wave amplitude decreased (especially at force levels of 20% MVC, the lowest levels looked at in their study) and attributed it to an impairment of neuromuscular transmission. Obviously, further work needs to be done in this area. A potential study would focus on the effects of prolonged firing on selected motor unit(s) and correlate changes in amplitude of MUTA to changes in amplitude of M-wave. This would permit an examination of changes in membrane properties of a motor unit versus the motor pool of the muscle. In addition, this experiment could answer the question of whether neuromuscular junction impairment arises from prolonged motor unit activity.

4.7 Conclusions

How do these experiments contribute to our understanding of the effects of prolonged motoneuron activity on motor unit behaviour? Referring back to Figure 1.2, we see that the motoneuron is an integrator which receives a variety of inputs: noise, descending commands, and afferent feedback. In this case, noise refers to spinal cord inputs to the motoneurons, excluding descending commands and afferent feedback. Noise does not include the noise of variability in the descending commands and afferent signals. The descending commands were monitored in these experiments using surface EMG. As expected, and as seen by most researchers, descending drive increases with prolonged constant force output. As the current model of motor control describes, this descending drive from the CNS controls the activity of a group of homonymous motor units rather than each motor unit being controlled individually. With such a model, the descending drive should act on each motoneuron in the pool in precisely the same way.

However, due to variations in their intrinsic properties, individual motoneurons within a motor pool may have different responses to a common input. Refer back to Figure 1.2, to inputs which modify motoneuron behaviour, feedback and noise signals. In fact, feedback signals may actually act presynaptically so as to change the amount of drive that the individual motoneuron receives. Noise input to the motoneuron is detected by changes in firing rate variability. However, variability in motoneuron firing may increase due to increased variability in the descending commands, increased variability in afferent signals, and other noise from undefined sources in the spinal cord. None were detected during either the constant rate or the constant force paradigms of the current experiment. At such rates of firing (~10Hz), motor unit firing rates do not appear to be affected by noise. At slower rates, however, firing rate variability is affected by preceding fatiguing activity. This is probably because at slower rates of firing (where ISI > 100 ms) the trajectory of the AHP is more sigmoidal (and less linear) in shape and is therefore affected more by noise (Jones, 1995; Matthews, 1996).

Since noise does not appear to have a great impact on motor unit behaviour and drive to all units of the pool is assumed to be equal, the greatest impact on a motor unit's response to prolonged firing must arise from the properties of the motor unit itself combined with feedback to the motoneuron. Given an increasing drive and a constant firing rate, muscle fibre and membrane properties were observed to change such that the amplitude of a motor unit triggered average of the EMG was greater after the activity. This would have been due to changes in ion conductances of the membranes. At the same time, the force generating system of the unit was also affected by prolonged firing because the twitch profile amplitude also increased. This increase was not due to synchronous motor unit firing. How and why would the twitch profile have potentiated following prolonged motor unit firing? First, the twitch potentiation could have been due to a release of surplus calcium. Second, the purpose of the potentiated twitch is to maintain the force output of the muscle. Twitch potentiation is observed during submaximal activation because the unit is not tetanized and therefore its output is not maximized. Then how is the potentiation of the twitch linked to the increase in amplitude of MUTA? Since the increase in twitch profile magnitude is linked to increased calcium release, either excitation-contraction coupling efficiency or the amount of calcium released must also be increased. One possible way of doing that would be to increase the degree of depolarization of the t-tubule membrane on the muscle cell, but to achieve this the muscle action potential magnitude must have also increased. Such an increase is reflected in MUTA. The magnitude of a muscle action potential is governed by sodium conductance through voltage gated channels. Presumably the increased sodium conductance is due to greater end-plate potentials. Perhaps this is attributable to greater amounts of acetylcholine released from the terminals of the motoneuron?

These changes in muscle unit membrane and mechanical properties appear to be task specific. Similar changes were not observed when the task was a force-defined protocol.

Evidence of a decline in the conduction velocity of muscle action potentials was seen in EMG power spectra, MUTA peak-to-peak width, and M-wave peak-to-peak width. Although a broadening of the peak-to-peak width of MUTA compared at the start and end of the period of constant force was the only parameter to attain statistical significance (p=0.01), the other measurements definitely showed evidence of similar trends and perhaps significance would be shown given additional data. As well, recall that all four M-waves (MVC and submaximal) broadened, as was measured by their peak-to-peak widths. A decrease in conduction velocity could be due to potassium accumulation in the t-tubules (Edman, 1995); however, if this were the case, one would expect to see consistent attenuation of motor unit twitches (Stuart and Enoka, 1992) which was often not seen during submaximal force protocols.

Throughout the discussion of these experiments, care was taken to distinguish between prolonged firing and fatigue. This is in part a result of problems with the definition of the term fatigue and the loose way in which it is often used. To clarify, for this study we assumed that fatigue does not automatically result from prolonged motor unit firing. Since most fatigue experiments were designed using maximal voluntary contraction protocols, the definition of fatigue was developed based on an inability to maintain an MVC. One of the definitions of fatigue given in Chapter 1, Section 1.3, is "an impairment in the ability to produce a maximal voluntary contraction." Fatigue may also result from prolonged muscle use at submaximal as well as maximal levels. The phenomenon of fatigue appears to involve a multitude of changes in the properties of the motor control system. Consider the motor unit twitch. With fatigue, the associated twitch amplitude of a motor unit decreases as a result of myofibrillar fatigue. However, as shown in these experiments, twitch amplitude may often potentiate following prolonged activation of a motor unit. This increase in twitch amplitude has also been observed by others in their fatigue experiments (for example, Bigland-Ritchie, Furbush, and Woods, 1986). A fatigued muscle is presumed to have fatigued muscle units, although a fatigued muscle unit, as has been shown, may or may not be one with an attenuated twitch profile. In any event, some researchers (Gandevia et al., 1995) solve the problem of too broad a definition by narrowing the definition to focus on only a reduction in the force generating or shortening ability of the muscle fibres.

Often, the broad definition of fatigue will not apply to what occurs during submaximal contractions. Two examples are muscle wisdom (Cresswell et al., 1996) and twitch potentiation. The muscle wisdom concept describes how motoneuron firing rates decrease to account for changes in muscle fibre contractile properties. The slowing of firing rates occurs only during maximal voluntary contractions. Consider also a sustained submaximal contraction: over the duration of the contraction the EMG to the muscle will increase but the MVCs measured before and after the contraction will not have changed at all. This example illustrates why care must be taken in the use and application of the term fatigue in the literature.

The results of this study suggest that additional work needs to be done to further examine changes in muscle fibre membrane properties before, after, and during prolonged firing. In doing so, adjacent motor unit activity should also be considered. An attempt should be made to correlate changes in MUTA's profile with those of the M-wave as well as how these changes reflect what is happening to the motor unit.

APPENDIX A

First Dorsal Interosseus

First Dorsal Interosseus (FDI, abductor indicis), a muscle which is functionally involved during movement of the index finger, is located on the dorsal surface of the hand between the thumb and index finger (Figure 1). As a bipennate muscle, FDI has two heads (a muscle head is also referred to as a point of origin). The largest head is attached along the lateral (radial) surface of the second metacarpal while the smaller head is attached to the proximal region of the medial (ulnar) surface of the first metacarpal bone (Gray's Anatomy, 1973). FDI inserts on the lateral side of the base of the proximal phalange of the index finger. Because FDI has two points of origin, there are two lengths of muscle fiber: fibers from metacarpal I are 1.6 cm in length while metacarpal II fibers range from 2.5 to 3.5 cm in length with an average of 3.1 cm (Enoka et al., 1989).

Johnson et al. (1973) reported the relative proportions of Type I and Type II (a and b) muscle fibre types in FDI in a study using the cadavers of five men between the ages of 17 and 30. They gave the mean percentage of Type I fibres as 57.4% (with 95% confidence the mean is 51.4 -63.4%) while that of Type II fibres was 42.6% (with 95% confidence the mean lies between 36.6 and 48.6%). In this study, fibre types were identified using myofibrillar ATPase staining techniques. Recall that Slow (S) twitch motor units have Type I muscle fibres and Fast (F) twitch motor units have Type II muscle fibres. Hence, given the lower innervation ratio of slow versus fast twitch motor units, FDI is innervated by more S than F-type motor units.

Offshoots of the deep palmar branch of the ulnar nerve innervate FDI. Prior to joining the ulnar nerve, efferents exit the spinal column via the eighth cervical and first thoracic nerves.

During abduction of the index finger, FDI is not only the prime mover, but it is also the only muscle to force the finger into abduction. As well, during flexion FDI plays the role of a synergist, that is, it assists other muscles in performing this task. The third role of FDI is that of a stabilizer during extension of the index finger. In consequence of its relative accessibility and the fact that it has two degrees of movement, FDI has been studied extensively by many researchers (Milner-Brown et al., 1973 a, b, and c ; Kranz and Baumgartner, 1974; Milner-

Brown and Stein, 1975; Desmedt and Godaux, 1981; Enoka et al., 1989; Fugelvand et al., 1993; Jones et al., 1994).

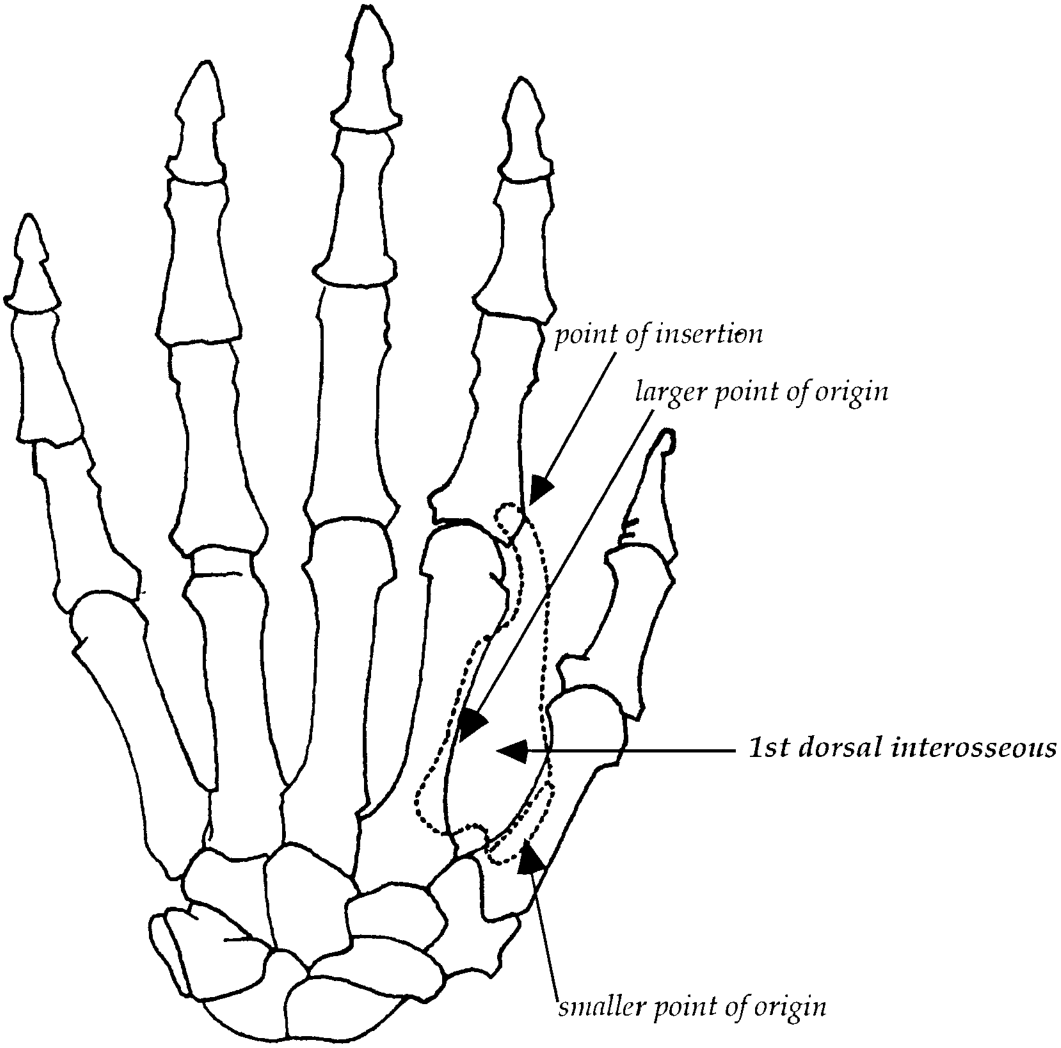


Figure 1. Bones of the left hand. Dorsal surface illustrating the location of First dorsal interosseus (FDI or 1DI).

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