EFFECT OF PROLONGED CONTRACTION ON PROPERTIES OF MOTONEURON AND MUSCLE MEMBRANE

by

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EFFECT OF PROLONGED CONTRACTION ON PROPERTIES OF MOTONEURON AND MUSCLE MEMBRANE

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ABSTRACT

Prolonged usage of the neuromuscular system is understood to result in reduced force output, which is termed fatigue. Reduction in force is due to changes in firing rates of motoneurons, changes at the neuromuscular junction and central fatigue, which have been studied before. No information on fatigue of human motoneurons exists. The primary purpose of this thesis was to examine fatigue of human motoneurons and associated changes in the motor units of first dorsal interosseous (FDI) muscle in humans. The FDI was chosen as it can be studied in isolation and a large body of literature exists on the neural control of this muscle. The fatigue of motoneuron was defined as the increase in excitatory input required by the motoneuron to discharge at a constant rate. Along with fatigue of motoneurons, we also examined changes in variability of the inter-spike intervals, power spectrum of muscle's electromyogram (EMG) and fluctuations in force while the subject maintained a constant firing rate of a targeted motoneuron under isometric conditions.

Single motor unit activity, surface EMG and force were recorded while the subject maintained a constant firing rate of a well identified motor unit from FDI muscle using visual and audio feedback for as long as possible (mean ± SD = 448.25 ± 226.65 seconds). All recorded motoneurons were categorized as fast firing or slow firing based on their firing rates.

The analysis revealed significant increase in EMG magnitude, indicating greater descending input to motoneuron to maintain constant firing rate. Statistical analysis of ISI showed increased variability indicating increased synaptic noise. Median power frequency decreased indicating changes occurring at neuromuscular junction and/or sarcolemma. The magnitude of force fluctuations increased, which would support an increase in inaccuracies in force output after fatigue. Similar changes in variability of ISIs were observed in motoneurons that fired simultaneously with the targeted motor units.

DEDICATION

To my creator, who gave me life so that I could achieve my dreams,
to my parents who have always believed in me,
to my dear sister,
to my friends who were there to cheer me up through the ups and downs
of life and those few wonderful teachers who have
shaped me into what I am today.

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ABBREVIATIONS

σ	Sigma (standard deviation)
μ	Mu (mean)
AC force	Bandpass filtered DC force depicting changes in force fluctuations
Ach	Acetylcholine
ADP	Adenosine diphosphate
AHP	Afterhyperpolarisation
AMP	Adenosine monophosphate
ATP	Adenosine triphosphate
AMIC	Average measure intra-class correlation
Ca^{2+}	Calcium ions
CNS	Central nervous system
CoVar	Coefficient of variation
DC Force	Constant force recording
EMG	electromyography
FDI	First dorsal interosseous
FFT	Fast fourier transformation
Hz	Hertz
Imp/s	Impulses per second
IS	Initial segment
ISI	Inter-spike interval
K^+	Potassium ions
KHz	Kilo hertz
Mn	Motoneuron
MPF	Median power frequency
MUAP	Motor unit action potential
MVC	Maximal voluntary contraction
M-wave	Compound muscle action potential
Na^+	Sodium ions

NMJ	Neuromuscular junction
PCM	Pulse code modulated
SD	Soma dendritic
SMIC	Single measure intra-class correlation
SMU	Single motor unit
SR	Sarcoplasmic reticulum
STA	Spike triggered averaging
TMS	Transcranial magnetic stimulation
TTL	Transistor transistor logic pulse
T-tubule	Transverse tubules

INTRODUCTION

The human neuromuscular system is involved in performing a wide range of complex motor functions. Such complex motor functions, when performed for prolonged periods, lead to complex changes in the central and peripheral elements comprising the motor system. In general, these effects of prolonged usage have been assessed in terms of fatigue, which has traditionally been defined in terms of decrease in force. Inaccuracies and instabilities in motor output accompany fatigue. A number of factors at various levels of the neuromuscular system contribute to the failure of appropriate motor output. For example, a decrease in descending cortical inputs to the motoneuronal pool (Bellemare and Bigland-Ritchie, 1987), decreases in firing rate of the motoneuron during prolonged firing (Bigland-Ritchie et al., 1983), failure of propagation of the neuronal impulse through the neuromuscular junction (Johnson and Sieck, 1993), failure of propagation of an impulse across the muscle membrane (Krnjevic and Meledi, 1958), failure of the excitation-contraction coupling mechanism (Edman and Lou, 1990), and failure of the contractile machinery, have all been reported.

Various research reports have highlighted the sites where fatigue is suggested to occur in the motor system, some of which are illustrated in Fig. 1. There are differences of opinion on the contributions of various sites to fatigue. Some of these differences may lie in the differences in experimental preparations and species of animals used for experiments. Such differences will be brought out in the review of the literature provided below. Since this thesis deals with the properties of motor units, a brief description of the motor unit precedes the review of literature on fatigue.

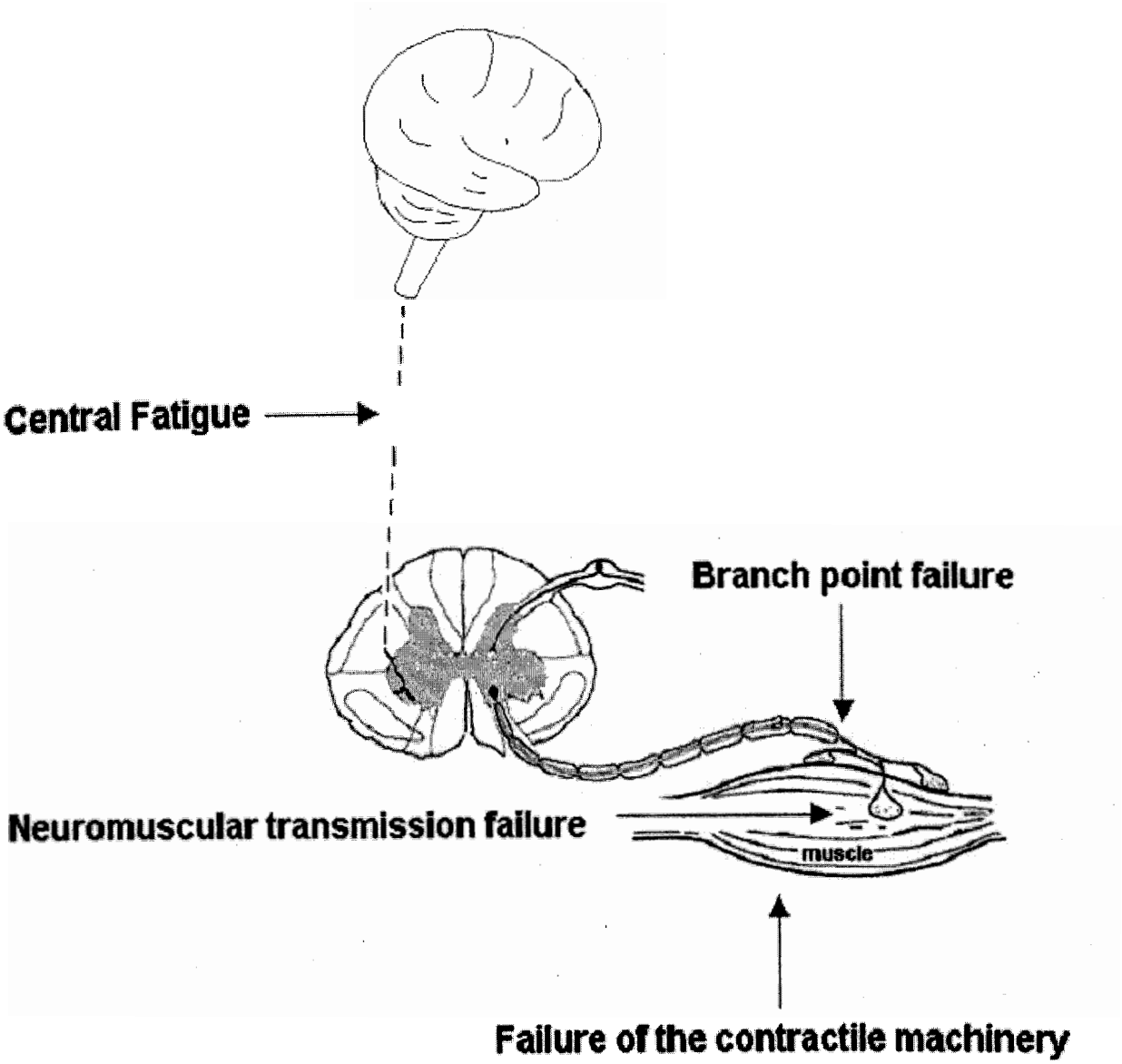

Figure 1: Figure shows various sites of fatigue, where fatigue related changes alter normal functions of the neuromuscular system.

Motor unit

Structure

Muscle fibres are innervated by motoneurons, the cell bodies of which are located in the grey matter of spinal cord for limb and trunk muscles. Each motoneuron has one large axon, which before innervating the muscle, branches several times to produce terminal arbourizations. Each terminal branch innervates a single muscle fibre. A

motoneuron plus all muscle fibres innervated by it comprise a 'motor unit'. All muscle fibres innervated by one motoneuron form a 'muscle unit' (Burke et al. 1973). In a normal healthy adult mammal, a single muscle fibre is innervated by one and only one motoneuron. Total number of muscle fibres innervated by a single axon is termed as innervation ratio. In a particular muscle, this ratio is greater for motor units with larger motoneurons in comparison to smaller motoneurons. Innervation ratios between large and small muscles cannot be compared. Muscle fibres innervated by any single motoneuron cover a considerable cross section of the muscle, which also aids in the production of uniform force.

Motor unit discharge characteristics

When a motoneuron is excited by a short excitatory input (< 2 ms), a spike is produced at the initial segment, which is called initial-segment (IS) spike or 'A' spike (Calvin and Schwindt, 1972). While A spike travels to the axon terminal to excite the muscle, it also excites the motoneuron soma and brings it to threshold. The soma discharges and produces soma-dendritic (SD) or 'B' spike. The B spike is followed by a long afterhyperpolarization (AHP). Sometimes, the B spike is followed by a delayed depolarization; if this delayed depolarization is large, a second spike follows the first spike, producing a doublet. This doublet is followed by AHP that has twice the duration of the original spike. Motoneurons innervating slow contracting muscle fibres, have long AHPs while motoneurons innervating fast contracting muscle fibres have shorter AHPs (Kernell, 1965b; Person and Kudina, 1972). When a motoneuron is depolarized for periods longer than the duration of AHP, it discharges rhythmically. The rate of firing

depends on the level of depolarization. With currents injected in cat motoneurons it has been shown that greater the level of depolarization, the higher is the firing rate (Kernell, 1965b). In intact preparations, such has human subjects, the same result holds. Instead of input current one estimates the input to the motoneurons from the force output. In general as long as the current stays constant, the inter-spike interval should remain constant. However there are several mechanisms, that alter inter-spike interval (ISI) even when current is kept constant. One of the perturbing factors is the membrane noise. The motoneuron membrane is constantly bombarded with random excitatory and inhibitory synaptic inputs resulting in a noisy membrane voltage. The membrane voltage fluctuates around a mean; the amplitude of the noise can be as large as 10 mV in the cat spinal motoneurons (Calvin and Stevens, 1967; 1968). Due to this noise, the membrane depolarization fluctuates and so does the instantaneous firing rate of the neuron. The firing rate is frequently expressed in terms of ISI. Without noise, the ISI has a constant value depending on the level of membrane depolarization, the higher the depolarization, the shorter the ISI. In the presence of noise, inter-spike interval varies around a mean value. At high firing rates, the ISIs follow a Gaussian distribution (Person and Kudina, 1972); but at lower firing rates the intervals show greater variability (Person and Kudina, 1972; Jones and Bawa, 1997) and is skewed to fit a Gamma distribution (Stein, 1967).

Motoneuron adaptation

When the motoneuron is injected with a step current, it responds with a very high initial firing rate followed by a decrease in firing rate, which then reaches a steady state firing level (Kernell, 1965a; Sawczuk et al., 1995). This decline in firing rate at a

constant input current is called 'adaptation', and is an inherent property of a motoneuron (Kernell, 1965a,b; Kernell, 1983). Some motoneurons adapt quickly while others adapt at a slower rate (Kernell, 1965a,b). Increasing or decreasing the rate of change of stimulation current could alter the rate of adaptation; the greater the slope of input current, the greater is the adaptation (Baldissera, 1982). The pattern of adaptation for prolonged periods was initially studied by Kernell and Monster (1982a,b), and has recently been described in greater detail by Sawczuk et al. (1995). The total period of adaptation was divided into three phases. The amount of adaptation that occurred between the first two seconds, accounted for about 90% of the decrease in firing rate; it was defined as 'initial' adaptation (Fig. 2). The next phase was called 'early' adaptation and it occurred during the 2 to 26-second period. The third phase, between 26-second to the 60-second period, was called 'late' adaptation. Sawczuk et al. (1995) proposed that the initial adaptation prevented excessive discharge of motoneuron, while late adaptation was suggested to match progressive decrease in motoneuron discharge to increased motor unit contraction time. These matched changes in motoneuron discharge and muscle unit contraction time are suggested to optimize force production during fatiguing conditions (Kernell and Monster, 1982b; Bigland-Ritchie and Woods, 1984).

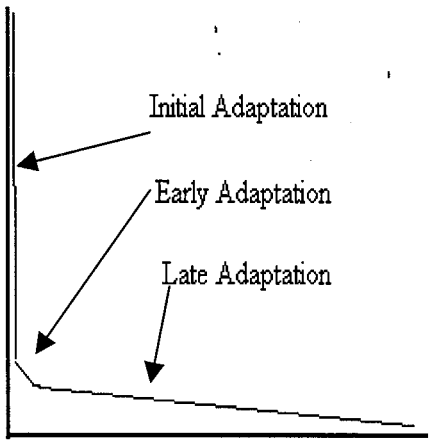

Figure 2: The three phases of adaptation of motoneurons that were identified during the repetitive firing of rat hypoglossal motoneurons in response to injected step currents (Sawczuk et al., 1995).

The adaptation observed in cat motoneurons with fast injected currents would be extremely difficult, if not impossible, to observe in human experiments. It would be impossible to produce infinitely fast and sustained contractions to study associated changes in firing rate of motoneurons. Smith et al. (1995) examined effects of sufficiently fast contractions on adaptation of firing rate of human motoneurons. Adaptation was shown to depend on the rate of rise of force output. The higher the rate of change of force, the greater was the initial firing rate and greater the adaptation. The initial fast firing rates, produced at the onset of contractions, was proportional to the rate of change of force, and steady-state firing rate was proportional to the magnitude of contraction.

The ionic currents contributing to initial and late adaptation are proposed to be different (Sawczuk et al., 1995). The initial phase of adaptation is suggested to prevent excessive and wasteful discharge. The late phase of adaptation is believed to match the

progressive increase in twitch contraction times, which would allow motor units to maintain the required force production despite changes in firing rates. Inactivation of Na^+ channels are suggested to be responsible for the late adaptation. The mechanisms responsible for late adaptation will be discussed in relation to our results.

Recruitment and rate coding

A muscle is innervated by a group of motoneurons called the motoneuron pool. Motoneurons and motor units vary in size, fatigability, force output and rate of force production. One can recruit one or more motor units depending on the amount of force required. With the minimal ramp excitatory input to a motoneuron pool, a single motoneuron is recruited which discharges at a low rate. The first recruited unit is always the smallest unit. As the excitatory input is increased, the first motoneuron increases its firing rate, while the next larger unit is recruited (Henneman, 1965b; Milner-Brown et al., 1973b). Discharge of additional units is called recruitment, while the increase in firing rate is called rate coding (Milner-Brown et al., 1973b). The size related recruitment was first demonstrated in cats by Henneman (1957; 1965a) and in humans by Milner-Brown et al. (1973a), and is illustrated in Fig. 3. Henneman, with his systematic studies, clearly established the size principle of motoneuron recruitment. He and his colleagues showed that motoneurons are recruited in order of size; the smaller one is always recruited before the next larger one. This recruitment pattern by size and rate coding results in smooth force output and minimizes fatigue.

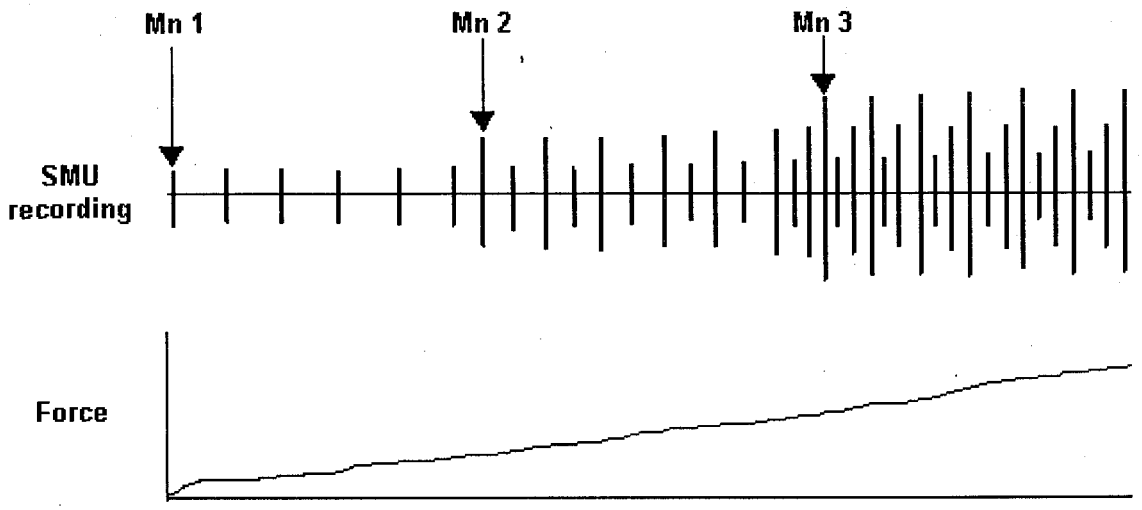

Figure 3: Top trace of figure shows SMU recording and lower trace shows force. With minimal excitation, a small single unit fires. With increase in current intensity, additional motor units are recruited as indicated by Mn 2 and Mn 3 (Mn stands for motoneuron).

Force output

A single action potential produced in the motoneuron results in producing a motor unit twitch, which is a biphasic response with a contraction phase and a relaxation phase. During rhythmic firing of a motoneuron, twitches start to fuse to produce partially fused contractions (partially fused tetanus). Each partially fused contraction has a DC (or constant) force component with a ripple superimposed on this constant force. The higher the DC component, the smaller is the ripple. When the firing rate increases to a value such that the ISI is shorter than twitch contraction time, the force output has no ripple and is said to be tetanized. Under normal physiological conditions, motor units fire at rates that produce partially fused contractions.

During a physiological contraction motoneurons fire asynchronously with respect to each other as shown in Fig. 4. Each produces its own partially fused force with different amount of DC component and magnitude of ripple. The magnitude of DC

component and the ripple depend on the size of the muscle unit, its contraction time and the firing rate. Since motoneurons fire independent of each other, the peaks and troughs of different ripples superimpose to produce 'an almost' smooth force output at the tendon as shown by the top trace in Fig. 4. However, it should be noted that the net force output of the muscle is never that smooth. It always has a small amount of ripple in the net force. This ripple in the net force output shall be referred to as the AC component of the force. Changes in the magnitude of the AC component during prolonged contraction time were assessed in this thesis.

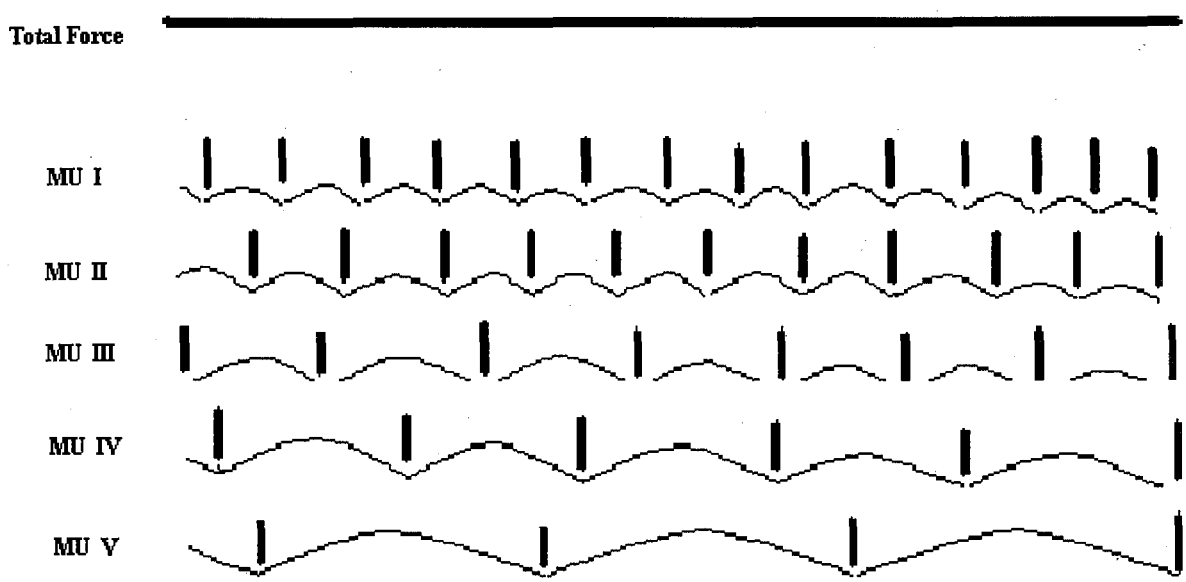

Figure 4: Figure illustrates firing of five different motor units. Their spikes are asynchronous with respect to each other. The vertical lines represent action potentials or spikes while the wavy lines represent the partially fused tetanic force of the corresponding muscle unit. The top trace is the net force that is produced as a result of asynchronous firing of the various motor units.

Fatigue

The force output of the muscular system can decline due to changes occurring at different sites in the neuromuscular system. A short survey of the literature dealing with some of these sites is discussed below. Even though this thesis did not deal directly with

changes in force during prolonged firing, the knowledge of mechanisms and sites of fatigue reported in the literature will be relevant to the discussion of our results dealing with prolonged contractions.

Central fatigue

Under normal conditions when a subject intends to make a voluntary movement, signals are received at the motoneuron pool from the CNS. Depending on the strength of the net excitatory signal from the CNS, recruitment of motoneurons occurs (Freund, 1983). When a subject decides to execute a maximal voluntary contraction (MVC), a strong input signal is sent from the cortex to the motoneuron pool, which causes recruitment of all motoneurons to produce maximum output from the corresponding muscle. This strong maximal signal cannot be maintained for long and one of the reasons is that the motoneuron pools receive less signal. This decreased signal to the pool is said to result from central fatigue. No matter how much psychological effort the subject exerts, the motoneuron pool cannot be driven maximally. Such decreases in drive resulting from fatigue of the central structures have been reported by several authors (Bigland-Ritchie et al., 1978; McKenzie et al., 1992; Gandevia, 2001). Bigland-Ritchie et al. (1978) defined central fatigue as the decrease in voluntary activation of all motoneurons in the motoneuronal pool when subjects performed a maximal voluntary contraction.

To test for central fatigue, one needs to show that the decrease in force is not due to peripheral factors; it is the motoneuron pool and hence the muscle that is not being driven maximally. Merton (1954) used a "twitch interpolation technique" to demonstrate

the absence of central fatigue on human subjects performing MVC of flexor pollicis longus muscle. While the subject ' exerted maximal voluntary effort, Merton superimposed an electrical stimulus of the muscle nerve on the voluntary activity. If the decline in force during fatigue was due to a decline in central command signal, the electrical stimulus should have produced extra force. Merton reported an absence of additional force with the electrical stimulus. From this observation he concluded that although the motoneuron pool was being driven maximally, the peripheral neuromuscular system was not capable of maximal output; that is, the decline in force is peripheral in origin. Later studies by other scientists have shown contrasting results. Bigland-Ritchie (1978), using the twitch interpolation technique on subjects performing 60-s of MVC of the quadriceps muscle, observed a decrease in force produced during maximum voluntary contraction but not when the muscle was stimulated electrically at 50 Hz. This indicated that the muscle was still capable of producing maximum force and the fatigue was central in origin. McKenzie et al. (1992) reported similar results using MVC of human elbow flexors and diaphragm. Using sub-MVC (50% MVC), Bigland-Ritchie et al. (1986b) were able to find evidence of central fatigue only in soleus and not in the other muscles tested (quadriceps and adductor pollicis). The results appear to be different for MVC and sub-MVC contractions.

In recent years, scientists have used trans-cranial magnetic stimulation (TMS) to study central fatigue. Taylor et al. (1999) studied changes in the cortically stimulated evoked responses and compared these to peripherally stimulated evoked response in human subjects on the biceps brachii and brachioradialis muscles. These authors

reported a decrease in excitability of the motor cortex indicating fatigue at the motor cortical level.

From the literature, the possibility of changes in the descending input or a decrease in neuronal drive to the motoneuronal pool is well supported during MVC. Though this view contrasted with Merton's earlier work in 1954, there is sufficient evidence to accept the existence of central fatigue. The data are difficult to interpret for sub-MVC contractions. In general, the EMG studies show that descending signals increase as fatigue sets in (Bigland–Ritchie et al., 1986b). It is after considerably long periods that EMG finally decreases.

Changes in motoneuron

Changes in motoneuron firing rate during maximal voluntary contraction (human experiments)

During maximum voluntary contractions, the motoneuron exhibits a decrease in rate of discharge with fatigue (Bigland-Ritchie et al., 1983). This decrease is suggested to result from decreased input from descending pathways (McKenzie et al., 1992), or a decrease in the net excitatory input from reflex pathways (Bigland-Ritchie, 1986a; Woods et al., 1987), or also due to changes in the inherent properties of motoneurons at the axonal level (Vagg et al., 1998; Kuwabara et al., 2001). The decrease in firing rate is accompanied by a significant decrease in EMG. A decrease in EMG leads to decrease or loss of force production. The decrease in firing rate was considered to be neural in origin since electrical stimulation of the muscle showed that the muscle was not fatigued (Bigland-Ritchie et al., 1978). Neural factors include both descending and reflex inputs

to the motoneuron pool. Taylor et al. (2000) observed supraspinal fatigue of the motor pathways on human subjects using the transcranial magnetic stimulation technique, giving support to the fact that descending inputs decrease during fatigue.

The contribution of changes in reflex feedback during fatigue has also been investigated. It was shown that reflex inhibition arising from group III and IV increases during fatigue (Bigland-Ritchie, 1986a; Woods et al., 1987; Garland, 1991). Macefield et al. (1991) showed a decrease in excitatory feedback from spindles of a fatigued muscle. In summary, the excitatory input to motoneurons decreases, the inhibitory input increases; the combination of the two decreases motoneuron firing rate during fatigue. Overall, both reflex and descending inputs seem to decrease motoneuron firing rate during an effort to maintain MVC. The inherent property of the motoneuron that leads to a decline in motoneuron firing is adaptation. It will be pointed out in the Discussion that adaptation is not fatigue. Adaptation codes for rate of change of current input and subsequent coding for rate of change of force output. It does not reflect upon any impairment or "fatigue" of the motoneuron function.

Changes in the motoneuron firing rate during sub-maximal voluntary contraction (human experiments)

Discussion of sub-maximal contractions will be discussed in detail because the thesis project has dealt with sub-maximal voluntary contractions of first dorsal interosseous muscle. The effects of sub-maximal voluntary contractions have been shown to contrast with the observations made with MVC. Some authors have reported an increase in firing rate during sub- MVC contractions (Maton and Gamet, 1989; Fallentin

et al., 1993), while others have reported a decrease in firing rate (Person and Kudina, 1972; DeLuca et al., 1996; Conwit et al., 2000), or no changes in firing rate (Maton and Gamet, 1989). Person and Kudina (1972) studied changes of rectus femoris muscle on normal human subjects. They reported a decrease in firing rate when the subject maintained a sub-maximal voluntary contraction at a constant force (approximately 17-35 % MVC for the different subjects). Recruitment of additional motor units was also observed, which occurred in order to maintain the target force output. DeLuca et al. (1996) reported similar decreases in firing rates while studying the tibialis anterior and first dorsal interosseous muscles of normal human subjects. Subjects performed a sub-MVC ranging from 30 % to 80 % MVC. Since the voluntary contractions were held for short periods of time (30 seconds), recruitment of new motor units was not observed during constant force contractions and moreover authors have not reported changes in EMG. Conwit et al. (2000) studied the quadriceps muscle of normal human subjects using 10 – 30 % MVC, and reported a decrease in firing rate of the motor unit along with an increase in the surface EMG. An increase in EMG implies recruitment of additional motor units. All these studies reported a decrease in firing rate when force was held constant. In the three studies, mostly the muscle contractions were held at lower levels of force under normal behavioural conditions, barring the study by DeLuca et al. (1996) where contractions of 80% MVC were also used.

In contrast to the above reported studies, Maton and Gamet (1989) reported increased or stable firing rates of motor units in their experiments on the biceps brachii and brachoradialis using 20 – 30 % MVC on normal human subjects. Fallentin et al. (1993) reported similar results performed on human biceps brachii muscle using 10 %

MVC. This difference compared to the other studies reported above could probably be due to the difference in the initial firing rates. In the above mentioned studies, DeLuca et al. (1996) reported initial firing rates of 20 – 30 imp·s^{-1} (impulses per second) in most trials, and Person and Kudina (1972) reported 15 – 19 imp·s^{-1} in most trials, while firing rates of 11 – 15 imp·s^{-1} were recorded by Maton and Gamet (1989). The type of motor units recruited (high/low threshold) might also have caused some of these differences, which has not been mentioned by these authors. Differences in the type of muscle, namely, fast versus slow or postural versus small hand muscle, were not the factors. A decrease in firing rate is observed in the first dorsal interosseous, which is a small muscle for fine control of movement. A decrease was also observed in large postural muscles, the quadriceps and the tibialis anterior. Biceps brachii falls in this latter category. Therefore it is suggested that the decrease or increase in firing rate with fatigue may have depended on initial firing rate rather than on the type of muscle.

Based on the results discussed from various literatures, it is evident that firing rate patterns changes with percentage of MVC used. Analysis of data from sub-MVC becomes very difficult due to changes in firing rates. However, if the firing rate is kept constant then changes in other parameters can be monitored and analysed in isolation.

Changes in variability of ISI

Under normal conditions a motoneuron discharges with a mean inter-spike interval μ. Due to membrane noise ISI shows variability; as ISI increases so does the variability (Person and Kudina, 1972; Jones and Bawa, 1997). During fatigue experiments at constant force, an increase in variability in ISIs has been reported (Person

and Kudina, 1972; Gantchev et al., 1986; Enoka et al., 1989; Sturm et al., 1997). For example, for first dorsal interosseous, Enòka et al. (1989) showed a significant increase in mean ISI, from pre-fatigue ISIs ($\mu \pm \sigma$: 75 ± 37 ms) to post-fatigue ISIs ($\mu \pm \sigma$: 112 ± 81 ms). This increased variability in ISI under constant force conditions is difficult to interpret, because an increase in variability was accompanied by an increase in ISI. From these experiments it is difficult to say whether increase in variability was due to an increase in noise or if it was due to an increase in ISI. On the other hand, Nordstrom and Miles (1991a,b) in their experiments kept the firing rate constant and still observed an increase in ISI variability in masseter motoneurons located in the brain stem.

Other changes in properties of motoneurons, which have been reported to occur during fatigue, are increase in firing doublets and synchronisation of firing among different motoneurons. At the onset of a slow contraction, some motoneurons fire doublets (Bawa and Calancie, 1983). A doublet has been suggested to result from the existence of delayed depolarization following a B spike. Griffin et al. (1998) observed a significant increase in number of doublets with fatigue in triceps brachii muscle. These doublets are also suggested to maximize force output (Thomas et al., 1999). Another property that is affected by fatigue is synchronisation of motor units. During normal voluntary contractions each motoneuron fires at sub-tetanic rates, and fires asynchronously. However, when carefully analysed, there is a small amount of synchronisation between motoneurons of a pool (Nordstrom et al., 1990). The index of synchronisation has been reported to increase with fatigue (Arihara and Sakamoto, 1999; Kleine et al., 2001).

Axonal conduction and neuromuscular junction

Under normal conditions, the action potential is triggered at the axon hillock of the motoneuron. Once triggered, the action potential is propagated down the axon to the terminal arborisations, where every terminal branch is invaded by an action potential. As a consequence, every muscle fibre innervated by the motoneuron is excited. All muscle fibres of a motor unit contract almost synchronously. During tonic firing of a motoneuron, the sodium-potassium pump is slowed and the axon hyperpolarizes (Vagg et al., 1998; Kuwabara et al., 2001) that causes slowing of axonal conduction velocity and may also cause branch point failure, near the terminal arborisation. Consequently, every muscle fibre may not be excited by the motoneuron. Across the neuromuscular junction, there is accumulation of K^+ and Na^+-K^+ pump may also slow down. This will affect the conduction speed along the sarcolemma. In extreme cases this slowing may cause failure of transmission of an action potential, and hence, failure of excitation-contraction coupling. Neuromuscular transmission failure has been reported in animal experiments using electrical stimulation. One of the chief factors that determine the extent of neuromuscular transmission failure is the rate of motoneuron discharge or rate of motor nerve stimulation (Johnson and Sieck, 1993; Kuei et al., 1990; Aldrich et al., 1986). For example, Johnson and Sieck (1993) stimulated diaphragm muscle of sprague-dawley rats at different stimulation rates. At low rates, neuromuscular transmission failure was not significant, the decrease in force was primarily due to failure of the contractile machinery. When stimulation rates higher than 50 $imp \cdot s^{-1}$ were used, neuromuscular transmission failure had a higher contribution to the decrease in force. Such high rates at which transmission failure occurs are not observed under normal physiological

conditions. However, changes at the neuromuscular junction and sarcolemma have been reported in the literature (Stephens and Taylor, 1972). This will be discussed below under M-wave.

Muscle Fatigue

Muscle contraction is a required entity to produce voluntary movement. Outputs from the CNS excite the muscle through motoneurons causing muscle contraction. When this output (action potential) reaches the neuromuscular junction, it activates the synaptic vesicles that release ACh into the synaptic cleft. Once ACh is released at the neuromuscular junction, a normal functioning muscle fibre will be depolarized. The depolarization travels along the sarcolemma to depolarize the T-tubules, which trigger the release of Ca^{2+} from the sarcoplasmic reticulum (SR). Once Ca^{2+} is released, cross-bridges are formed and contraction occurs. Failure in contraction can occur due to improper excitation of the sarcolemma, depolarization of the T-tubules, problems with triggering of Ca^{2+} release, cross-bridge formation and force output. Details of impaired function at different stages are given below.

Changes in the sarcolemma

Changes in conduction along sarcolemma can be assessed by measuring motor unit action potential (MUAP), M wave, or by computing frequency composition of EMG. Each of these parameters are briefly discussed below.

Changes in motor unit action potential

Under normal physiological conditions when an action potential arrives at the axon terminal, the nerve excites all muscle fibres belonging to its motor unit. Summation of activity from all fibres of the motor unit results in the motor unit action potential (MUAP). At low activity one can record MUAP from surface activity. As the strength of activity increases, EMG becomes an interference pattern and individual MUAPs cannot be discerned. One can obtain MUAP of each active motor unit by recording surface EMG and individual motor unit spike by intra muscular electrodes. Each motor unit spike recorded with a microelectrode samples only a few muscle fibres of the motor unit and is not a true representative of true MUAP. One can use a microelectrode recorded spike to obtain MUAP, by spike triggered averaging of unrectified surface EMG. Due to the multiple nerve branches, each potential arrives at a different time and hence the summed potential will be broader and smooth edged unlike the motor unit spike. This extracted compound potential will provide information on both the pre-synaptic and post-synaptic factors affecting the profile of MUAP.

When the muscle is exposed to prolonged contractions, or when it is fatigued, a widening of this potential has been observed. Burke et al. (1973) reported an increase in duration of the MUAP of medial gastrocnemius muscle units of anaesthetized cats using 40 Hz electrical stimulation. Similar findings were reported by Sandercock et al. (1985) of the medial gastrocnemius muscle units of anaesthetized cats using 10, 40 and 80 Hz electrical stimulation. Sandercock et al. (1985) used three different frequencies of electrical stimulation to mimic results from a previous study and also to observe changes

in low and high frequency fatigue. They reported an increase in the duration of MUAPs with all three stimulation frequencies. Reports from both studies (Burke et al., 1973; Sandercock et al., 1985) suggest the increase in MUAP duration to be due to failure at terminal branches of motor nerves, neuromuscular junction, along the surface of the muscle fibre and along T-tubules.

In humans, only one author has reported changes in MUAP with reference to changes with fatigue. Conwit et al. (1999) observed an increase in area of mean surface detected MUAP at low (< 30 % MVC) and high (> 30 % MVC) constant force contractions on human quadriceps femoris. Conwit et al. (1999) suggested the reported increase in MUAP area to be due to recruitment of additional new motor units. In later research they (Conwit et al., 2000) reported an increase in surface detected MUAP amplitude on human vastus medialis muscle using 10 and 30 % MVC contractions. Such an increase in MUAP amplitude was suggested to be due to recruitment of larger motor units and synchronisation of motor units.

From the above literature, it can be concluded that both MUAP duration (reports from animal studies) and MUAP amplitude (reports from both animal and human studies) increase with fatigue. An increase in MUAP amplitude is possibly due to recruitment of additional motor units. The possible mechanisms that could cause an increase in MUAP duration are a decrease in action potential conduction velocity along the sarcolemma, a decrease in excitability of sarcolemma (Milner-Brown and Miller, 1986), and failure at the neuromuscular junction. Failure at the neuromuscular junction has not been shown to occur in human subjects under normal physiological conditions (Bigland-Ritchie et al., 1982; Kuwabara et al. 2001).

Changes in power spectrum and M - wave

Each action potential, of each active motor unit contributes to EMG. Since each motor unit fires independent of the other motor units, peaks and valleys of different motor units overlap; so surface EMG is a smoothed version of single motor unit activity. When surface EMG is Fourier transformed, one obtains the power spectrum of EMG. Generally, the spectrum is a unimodal curve with the dominant peak around 100 Hz. There is very little power beyond 3 KHz. In the literature, instead of the peak frequency, mean or median power frequency (MPF) is used to indicate properties of the spectrum. During fatigue, if motor unit action potential slows down (broadens), it leads to lowering of the MPF. Firing rate also contributes to power spectrum by adding power at lower frequencies. Recruitment of new units adds to higher frequencies. Therefore, the shifts in MPF depend on the shapes of newly recruited units, firing rate of already and newly recruited units, intra-muscular temperature and slowing of the sarcolemmal action potential.

Petrofsky et al. (1979) showed an increase in the median power frequency when their subjects performed a VO_2 max test on a bicycle ergometer. Petrofsky had also noted an increase in median power frequency was observed at different sustained isometric force levels (20, 40, 60, 80 and 100 % MVC). Greater increase in median power frequency was observed at low force levels (20 and 40 % MVC) than at higher force levels (80 and 100 % MVC). Such an increase in median power frequency was proposed to result from an increase in recruitment of new motor units and due to an increase in the temperature of the muscle. In a later study Petrofsky et al. (1982) used

fatiguing sub-maximal isometric voluntary contractions of handgrip muscle group, biceps brachii, adductor pollicis and quadriceps' muscle at 25, 40 and 70 % MVC on normal human subjects. The power spectrum analysis showed a significant decrease in the median power frequency while force was held constant at the three levels. This decrease in median power frequency occurred even when additional recruitment was observed. Hence results can differ even from the same laboratory depending on the experiment protocol. Jensen et al. (2000) studied changes in power spectra on 8 normal human subjects when the subjects performed isometric shoulder abduction at 11 – 12 % MVC for a period of 30 minutes. Power spectrum analysis revealed no change in the MPF. Jensen and colleagues proposed that the lack of change could be due to recruitment of new motor units. That is, the lowering of MPF due to fatigue (slowing of action potential) was compensated by recruitment of new units that contributed higher frequency content to the spectrum. In summary, different authors have reported different results such as an increase, no change or a decrease in MPF in response to sub-maximal fatiguing voluntary contractions.

Changes occurring at neuromuscular junction, both pre and post-synaptic can also be assessed by examining M-wave before and after fatigue. A supra-maximal stimulus to the muscle nerve results in almost synchronous firing of all motor units. The recorded potential from this electrical activity is called M-wave. A muscle fibre action potential can become wider as a result of slowing of conduction velocity of muscle fibre, or change in dynamics of the transmitter. Widening of a muscle unit action potential could also occur if a motor axon slows down and the action potential arrives at the various terminal boutons with more time variability. With fatigue all these mechanisms contribute to a

widening of motor unit action potential. All these changes lead to a change in amplitude and duration of an electrically evoked M-wave. The same factors also affect MPF as discussed above. Merton (1954) used M-wave as an indicator to examine the extent of contribution of such factors to peripheral fatigue. In order to avoid contamination from neighbouring muscles, Merton used abductor pollicis muscle that is supplied by the ulnar nerve and it acts on the thumb. Fatigue was induced by MVC held for more than 3 minutes. No change or reduction in the M-wave amplitude was observed despite the significant loss of force in the muscle.

Merton's (1954) conclusion was well accepted until Stephens and Taylor (1972) showed significant decrease in M-wave area of first dorsal interosseous muscle. Subsequently, a number of studies have been performed to examine changes in M-wave. Some authors agree with Merton's conclusion (Bigland-Ritchie and Lippold, 1979; Milner-Brown and Miller, 1986; Belllemare and Bigland-Ritchie, 1987; McKenzie et al., 1992; Fuglevand et al., 1993), while others agree with Stephens and Taylor (Fournier et al., 1991; Bazzy and Donnelly, 1993; Fuglevand et al., 1993).

Failure of impulse propagation along sarcolemma

Under normal conditions, a muscle fibre contracts in response to an action potential. Action potential arising from the motoneuron reaches the muscle at the neuromuscular junction. Once the ACh activates receptors at the neuromuscular junction, an action potential is initiated, which spreads along the length of sarcolemma and down the T-tubules. When muscles are excited voluntarily for prolonged periods, Ca^{2+} ion levels tend to increase in the cytosol. Prolonged muscle contraction results in

failure of Ca^{2+} ion uptake by the SR (Lee et al., 1991). Increased Ca^{2+} ion levels in the cytosol causes ionic gradient changes, primarily with the Ca^{2+} dependent K^+ and Cl^- channels. As a result they tend to hyperpolarize thereby resulting in a decreased conduction velocity of action potential (Stephenson et al., 1995). Decreased conduction velocity may also result from a decrease in Ca^{2+} ion release from the SR due to voltage sensor inactivation in the T-tubules that results from repolarization or inactivation of the Ca^{2+} ion release channel (Lacampagne et al., 2000). These observed changes in conduction velocity are due to changes that occur in the muscle and not the neuromuscular junction (Bigland-Ritchie et al., 1982). Sjøgaard and colleagues (Bystrom and Sjøgaard, 1991; Sjøgaard, 1991) reported increased extracellular K^+ ion levels even after one hour after ceasing muscle activity (on subjects performing 10 and 20 % MVC static handgrip). Simultaneously, Lee et al. (1991) in their analysis reported elevated Ca^{2+} ion levels on the fatigued muscle fibres alone. Both these findings suggest that the decrease in conduction velocity in sarcolemma to be due to an increased Ca^{2+} ion levels and accumulation of K^+ ions in the extracellular space. Such an increase in accumulation is also known to occur due to increased acidity in the muscle (Light et al., 1994; Edman and Lou, 1990). In all cases discussed above, a significant reduction in action potential transmission occurs, which may alter force production.

Signal transmission from T-system to sarcoplasmic reticulum

Once the action potential is transmitted into T-tubules, Ca^{2+} is released from the SR. The release of Ca^{2+} enables the cross bridges to bind, thereby resulting in muscle contraction. In this chain, the T-tubules act as voltage sensors that trigger Ca^{2+} ion

release on sensing the action potential. Prolonged muscle contraction may compromise this function of the T-tubules. As a result of fatigue, K^+ ions accumulate in T-tubules and in turn reduce the effect of action potential in T-tubules (Hodgkin and Horowicz, 1959). Along with K^+ ion accumulation, a dysfunctional Na^+-K^+ pump may also be observed. These factors further limit the spread of action potential (Edman and Lou, 1992).

A compromised T-tubule will decrease action potential transmission to SR, thus resulting in decreased Ca^{2+} ion release. Decreased Ca^{2+} ion release decreases muscle fibre activity and in turn severely compromises muscle contraction, which is enhanced during fatigue resulting in severe force loss (Edman, 1995). Decreased Ca^{2+} also compromises cross-bridge function as it is required to bind with troponin C to aid in cross-bridge formation, thus failing to activating sufficient number of myofibrils (Lee et al., 1991). Due to these factors, an abnormal T-tubule would fail to excite the muscle, which results in excitation-contraction coupling failure, and thus reduced force output (Edman and Lou, 1990; Vollestad, 1997).

Failure of contraction

For a muscle to contract, two systems must be activated. Firstly, the muscle must be excited. Secondly, based on the level of excitation the contractile elements (myofilaments) should slide to produce force by forming cross bridges. Hence, excitation-contraction coupling failure may occur as a result of failure of excitation or failure of sliding mechanism of the contractile elements

It has been shown that the contractile machinery can perform work even after fatigue sets in. This is evident from results reported by Edman and colleagues (Edman,

1995; Edman and Matiazzi, 1981) in which they were able to potentiate a muscle twitch at a stage of moderate fatigue in a frog muscle fibre by administering caffeine (caffeine causes SR to release more Ca^{2+} ions). Guitierrez (1996) showed that skeletal muscle contraction could be potentiated despite an increased exogenous blood K^{+}, which indicates fatigue.

Contraction of muscle fibres is based on the sliding filament theory (Huxley, 2000). According to the theory crossbridges are formed between myosin heads and active binding sites in the actin filament. Actin slides over myosin to perform a shortening contraction. Two mechanisms aid in this function, 1) Ca^{2+} is required to bind to troponin C thereby exposing actin for crossbridge formation, 2) ATP is required to provide the energy required for binding to occur. Hence, abnormal Ca^{2+} release may result in poor function of the contractile machinery and poor ATP supply may also alter muscle function (discussed later). Beyond these two factors, fatigue or a decrease in force production may occur at the cross-bridges itself. Either a decrease in number of cross-bridge formation or the amount of force produced per cross-bridge could occur during fatigue. Measuring force loss along with changes in muscle stiffness assesses these factors. Edman and Lou (1991) measured both these factors, and reported poor cross-bridge turnover to be the primary cause of fatigue and not force loss per cross-bridge. A primary reason that may lead to cross-bridge failure is a decrease in Ca^{2+} ion release. Other biochemical changes that occur along with prolonged contraction of the muscle are discussed later.

There are various biochemical changes that cause contractile machinery failure. Broadly these changes are related to a decrease in energy supply and accumulation of

metabolites such as accumulation of lactic acid and inorganic phosphates. The latter is known to have a major contribution towards a decline in maximum muscle force, though the exact mechanism of how this happens has not been established (Cooke et al., 1988).

The subject of decreased energy supply has been one of controversy. Some scientists believe that there is inadequate energy supply (depletion of glycogen) in the muscle during prolonged usage, leading to fatigue (Balsom et al., 1999), while Grisdale et al., (1990) argues that the status of muscle (trained versus untrained) prior to any activity plays a greater role than glycogen stores in the muscle. Based on their work, Grisdale et al., (1990) concluded that fatigue is probably due to poor peripheral feedback or due to decreased descending neuronal drive. Other energy resource such as ATP, also serve a vital role since it is an important driving force behind muscle contraction. Miller et al. (1995) reported ATP depletion as a consequence of intense fatiguing exercises. However, this theory may not be acceptable in light of earlier work by Sahlin (1992). Sahlin (1992) proposed that the energy used in muscle depends on the type of activity and that ATP is generated in the muscle through both aerobic and anaerobic mechanisms. Each mechanism depends upon the availability of other factors, e.g., oxygen availability and inherent metabolic capacity of the contracting muscle fibre. These ATP generating mechanisms preserve the availability of ATP through feedback mechanisms. When the demand for ATP increases in the muscle, ADP and AMP accumulate and temporarily block activation of the excitation-contraction coupling (Sahlin, 1992). Having assessed the literature it is evident that inadequate energy supply may not be a significant factor in causing fatigue.

Other metabolic factors implicated in muscle fatigue are changes in muscle intracellular pH. During stressful exercise muscle pH could decrease (Sahlin, 1992). This is due to the accumulation of H^+ ions, inorganic phosphates and lactate from the breakdown of muscle glycogen. Accumulation of such metabolites leads to acidosis, which in turn inhibits ATPase enzyme activity (Edman and Matiazzi, 1981), and also inhibits muscle contraction (Miller et al., 1995). Such metabolite accumulation may affect excitation-contraction mechanism directly, by reducing cross-bridge turnover rates (Parkhouse, 1992), and may interfere with energy supply mechanisms.

In summary the evidence presented here, suggest these mechanisms play a greater role in muscle fatigue than previously thought. They affect the contractile machinery by reduction of the contraction and relaxation time by decreasing the speed of contraction and cross-bridge formation.

Conclusions

The above survey shows that observations reported in the literature vary considerably on changes in motoneuron, neuromuscular junction and the sarcolemma with fatigue. With slight changes in experimental paradigms from all the previous studies in the literature, we have re-examined changes in the structures and function of the motor unit, under more controlled conditions. Based on our results we have also tried to clarify some of these queries.

OBJECTIVES

Rationale

Previous research indicates fatigue or fatigue related changes occurring due to a few reasons a) decreased descending inputs to motoneuron or central fatigue, b) fatigue occurring due to changes at the neuromuscular junction and along sarcolemma and c) fatigue of the contractile machinery. Fatigue of the motoneuron itself has not been investigated.

Fatigue is well known to decrease force output, in turn also decreasing precision in performance. This decrease in precision can result from impaired spatio-temporal activation of various muscles involved in movement or may also involve impaired control of any one muscle. Such changes may occur due to additional noise in the motoneuron membrane, increased synchronisation of motoneurons, or under extreme conditions from failure of activation of some muscle fibres. This may result in large uncontrolled fluctuations in force instead of a smooth force output. We will examine some of these factors that could lead to a decline in precision using one muscle, the first dorsal interosseous (FDI). This muscle has been chosen as it can be studied in isolation by index finger abduction and moreover there is enormous literature available on the neural control of this muscle. The majority of motoneurons innervating first dorsal interosseous muscle are recruited at low levels; more than 50% are recruited below 10% of MVC (Milner-Brown et al., 1973a). Therefore, the force levels used in this study lie in the range 2 - 40 %. Higher force levels (> 10 %) were required for experiments with higher firing rates.

Using motoneurons innervating FDI muscle, this thesis aimed to gain an understanding of fatigue and fatigue related properties of motoneurons and associated changes in the neuromuscular junction and sarcolemma.

Objective

The primary objective of this thesis is to determine if motoneuron fatigues when subjected to prolonged firing under a constant firing rate protocol.

Hypothesis

I hypothesize that motoneurons fatigue when exposed to prolonged firing. Associated with the fatigue of the motoneuron are additional changes in the neuromuscular system. I further hypothesize that a fatigued neuromuscular system will demonstrate an increase in ISI variability and an increase in force fluctuations accompanied by changes occurring at the neuromuscular junction and sarcolemma.

Specific Aims

1. To determine if the net excitatory input to motoneuron increases with prolonged firing.
2. To determine if the inter-spike interval variability (statistical properties of the motoneuron) increases with prolonged firing.
3. To determine if there is a decrease in action potential transmission at the neuromuscular junction and along sarcolemma with prolonged firing.
4. To determine if force fluctuations increase with prolonged firing.

5. To determine if concomitantly firing motoneurons will exhibit parallel changes in their properties.

Research Plan

(1) Changes in descending input to a motoneuron in order to maintain constant firing rate

It has been shown in the literature that firing rate of a motoneuron declines and total surface EMG increases while force is maintained for prolonged periods. The decline in firing rate has been shown to result from a decrease in excitatory reflex feedback and an increase in inhibitory reflex feedback. What happens to the intrinsic properties of the motoneurons? Is there any change in channel currents or changes in firing threshold that could account for a decrease in firing rate? To answer this question we measured changes in EMG activity while subjects maintained a constant firing rate of the motoneuron. If motoneuron fatigues, it would require higher descending input to maintain the firing rate constant. This increase in descending input would in turn result in recruitment of additional fresh motor units which would be reflected as an increase in EMG activity after a prolonged firing.

(2) Statistical properties of a motoneuron

In previous studies an increase in the variability of inter-spike intervals has been shown both for constant force (Person and Kudina, 1972; Gantchev et al. 1986; Enoka et al. 1989; Sturm et al. 1997) and for constant firing rate (Nordstrom and

Miles, 1991a, b) protocols. While we were maintaining a constant firing rate of a motoneuron in our experiments, changes in variability of ISI were computed.

(3) Changes at the neuromuscular junction and the sarcolemma

From the review of literature above it was noted that there are a number of controversies over the changes in properties of motor unit action potential and mean/median power frequency of EMG power spectra. These parameters have not been computed during 'constant firing rate' protocol in previous literature.

In this study, profiles of the motor unit action potentials (MUAP) were computed by spike triggered averaging technique of unrectified surface EMG. The width and amplitude of MUAP were calculated to observe for any possible changes. Changes would reflect modifications occurring at the neuromuscular junction, and/or changes in conduction velocity along the sarcolemma. Increases in the jitter time in transmitter release or binding to ACh receptor increases will result in increased MUAP duration. Significant changes in these factors will also be reflected in the MPF of the EMG power spectra.

(4) Changes in force fluctuations

From the literature it is well known that variability of ISI increases in synchronization of motor units and increases in tremor occur during fatigue. Does the same thing occur when the firing rate of the motor units is maintained?

In this study the recorded DC force was band pass filtered to obtain AC force. Changes in magnitude of this AC force were computed.

(5) Changes in firing of targeted and non-targeted motor units

Nordstrom and Miles (1991b) reported to observe some motor units to increase and the other to decrease their firing rates while a targeted motor unit was maintained at a constant firing rate. During our 'constant rate' experiment we examined the behaviour of the non-targeted units. We examined if the firing rate of non-targeted units changed as reported by Nordstrom and Miles, (1991b) or remained constant as the targeted unit did. Furthermore, how did the coefficient of variation of non-targeted units change when compared to the controlled targeted unit?

METHODS

Experiments were performed on the first dorsal interosseous muscle of 5 normal subjects with no known neurological, neuromuscular or musculo-skeletal disorders. All subjects were males between 21 – 28 years. Prior approval for the experiments was obtained from the Research Ethics Board for Human Experiments at Simon Fraser University (see appendix A). All subjects were given a detailed explanation of the procedures prior to the experiments and each signed the informed consent form prior to the experiments.

Experiment procedures

To record activity from the first dorsal interosseous (FDI) muscle, the subject was seated comfortably with his shoulder slightly extended in order to position the forearm on a horizontal platform (Fig. 5). The forearm and the hand were kept in a prone position with the fingers extended. Velcro straps were used to stabilize and restrain the forearm to the platform. The lateral three fingers were strapped to each other and restrained with stops so as to minimize their contribution to the force measurements. Similarly the thumb was also restrained by an additional stop so as to help in isolating the action of the target muscle (Fig. 5). Force transducer for force and electrodes for electromyographic activity measurements were set up (see below for details) and the subject was given detailed instructions on how to proceed. The subject was asked to abduct his index finger isometrically against a force transducer and recruit a clear motor unit. Audio and visual feedback of the motor unit activity was provided to the subject. Once a clear motor unit

spike was observed it was quickly discriminated using a window discriminator. Now the subject received feedback of the only discriminated unit and was asked to increase its firing rate. When the experimenter determined that an appropriate rate was reached, the subject was asked to hold that rate as long as possible (up to 10 minutes). Such a controlled unit will be referred to as the "targeted" unit. The "appropriate" rate of the motor unit was determined by the voluntary effort that a subject could exert for 10 minutes, and/or the clarity of the unit could be maintained without the presence of additional units.

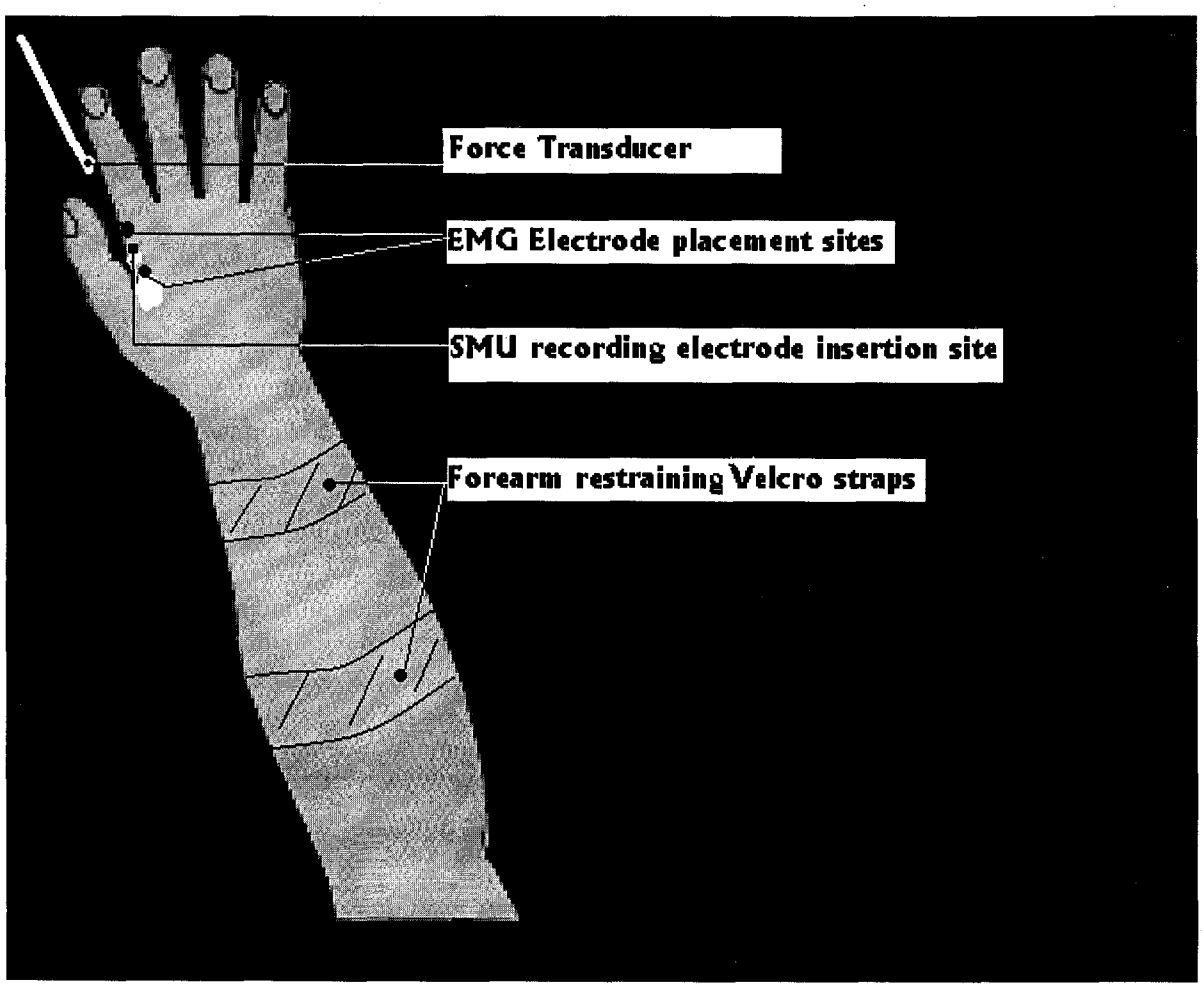

Figure 5: The figure shows placement of surface EMG electrodes on the first dorsal interosseous muscle and the site for the SMU electrode along with the positioning of the forearm during the experiments.

Each run lasted for approximately 10 minutes. If the first run consisted of a low threshold unit, the subject was asked to rest for 10-15 minutes, and a higher threshold unit was recorded. If the first unit required considerable force, the experiment was terminated after recording the first unit. Only two units were recorded during any one experiment. Experiments were performed at firing rates ranging from 7 to 15 $imp \cdot s^{-1}$.

Recording of single motor unit activity

The electrical activity of single motor units was recorded with a bipolar needle electrode. The intramuscular needle electrode was fabricated in the lab with the use of a 25-gauge needle carrying two insulated stainless steel wires, (California Fine Wire Company, Grover City, California, USA) 30 - 50 μm in diameter. Two wires were inserted into the barrel of a hypodermic needle such that the wire tips were at least two diameters apart. The electrodes were sterilised before use and each subject had his own electrode.

The two wires, which formed a bipolar electrode, were connected to a Grass P15 AC pre-amplifier (Grass Instruments Company, Quincy, Mass.) with a band pass filter of 100 Hz to 10 KHz and a gain of 100 for differential recording. The output from the pre-amplifier was sent to a conditioning amplifier to amplify the signals, the output of which was sent to an oscilloscope (Tektronix Oscilloscope) where the motor unit was monitored. In addition, the amplifier output was sent to other destinations. The first was the 1401 Plus computer interface for acquisition of data on the computer. The computer operated on Windows using SPIKE2 software from Cambridge Electronics Division (CED, Cambridge, U.K.). The raw motor unit activity was digitized at 13 KHz.

Ideally it should have been atleast 20 KHz, but the limitations of the system put 13 KHz as the upper limit. However, this frequency did not cause any problems in interpretation of our data as large sections were used for analysis. The second output from the amplifier was sent to a window discriminator made by BAK Electronics (Model RP – 1). Using voltage and time windows, the targeted motor unit was isolated to produce a TTL (transistor-transistor logic) pulse. The motor unit activity was also connected to a Grass AM – 8 audio monitor for audio feedback to the subject. In addition, the SMU activity was recorded on a video tape using a pulse-code-modulated (PCM) system (Model 4000A, Vetter, Rebersberg, U.S.A). The bandwidth of the channel recording single motor unit data was DC – 15 KHz. The schematic representation of the various connections is illustrated on Fig. 6.

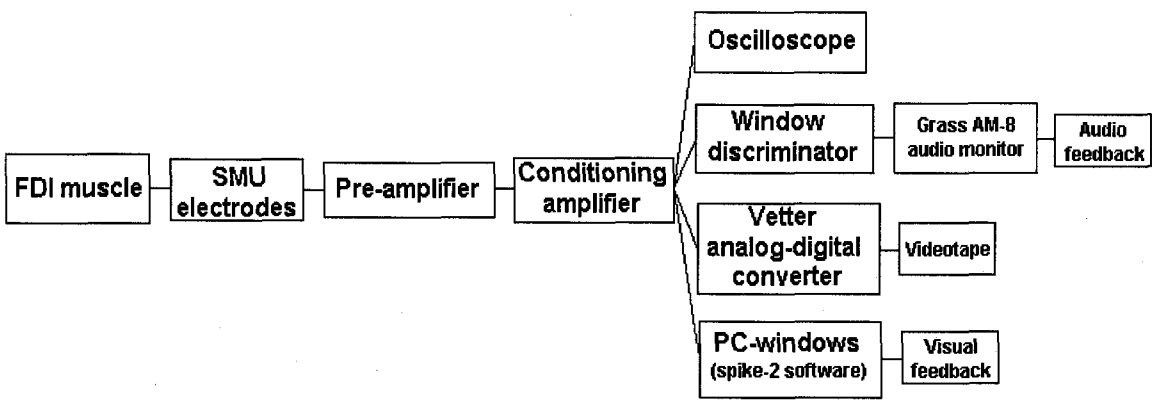

Figure 6: Schematic representation of the SMU recording.

Recording of surface electromyographic activity

Surface EMG was recorded using a pair of 9 mm Ag/AgCl cup electrodes that were taped to the skin (Fig. 5) overlying the first dorsal interosseous muscle. One electrode was attached over the belly of the muscle and the other over the tendon of

insertion at the base of medial aspect of the index finger. For better conduction, the skin was shaved (when needed) and scrubbed with alcohol. Electrode jelly was used to minimize resistance between the skin and the electrodes (Grass Instrument Company Electrode Cream).

The electrodes were connected to the Grass Pre-amplifier with a band pass filter of 30 Hz to 3 KHz and a gain of 100. The signal was further amplified and filtered. From the conditioning amplifier the signal was sent to three different destinations that included the oscilloscope, the Vetter Digital PCM recording adapter and the 1401 Plus interface.

EMG signals were acquired at 6.0 KHz using the 1401 Plus interface (Cambridge Electronic Design). The schematic diagram of the various connections is presented on Fig. 7.

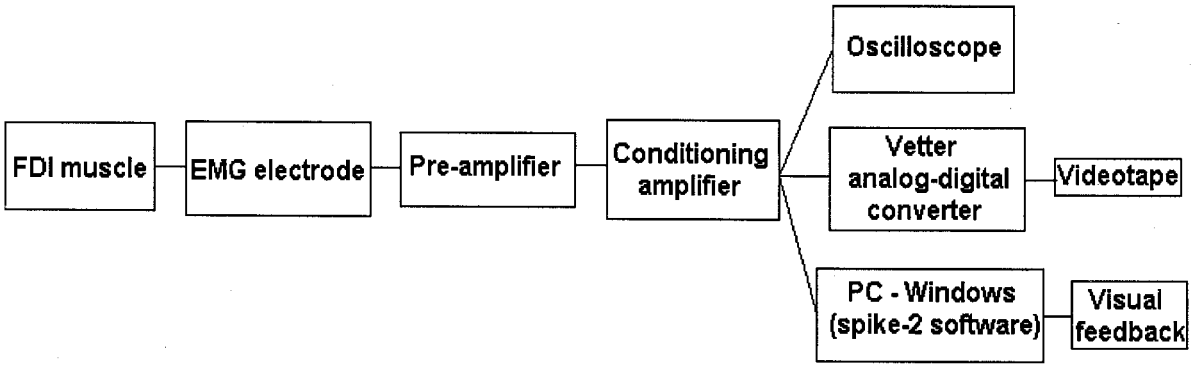

Figure 7: Schematic diagram of the EMG recording.

Recording of force

A Grass FT10 force transducer was used to record force of abduction exerted by FDI (a custom made lever with micro measurement strain gauges was used in initial few experiments). The transducer was placed against the proximal inter-phalangeal joint on

the medial aspect of the index finger (Fig. 5). The signals from FT10 were then sent through a bridge amplifier (Vishay Instruments, Model # 2310) to be amplified and filtered DC – 1.0 KHz. Output of the bridge amplifier was fed to the DC conditioning amplifier set at DC to 30 Hz band pass filter. These signals were recorded on videotape as above. Throughout the experiment the signals were monitored on the oscilloscope. DC force was digitized at a sampling rate of 50 Hz. The schematic diagram of the various connections is illustrated on Fig. 8.

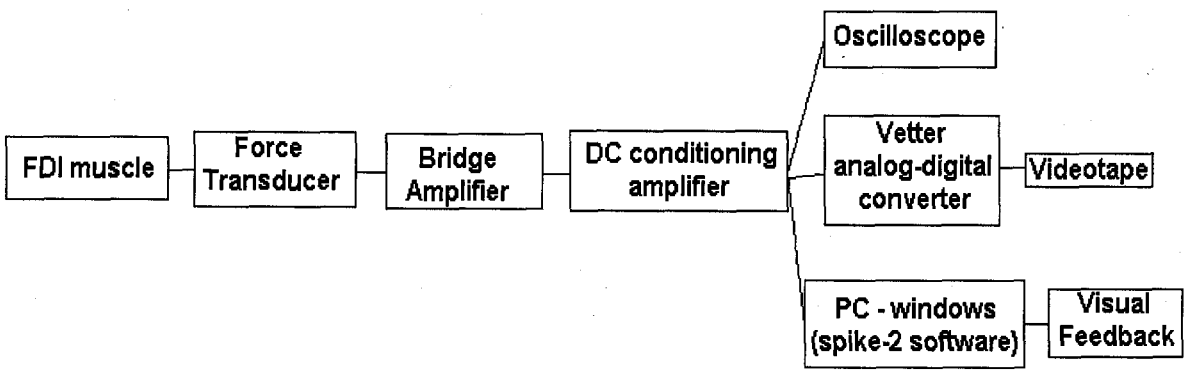

Figure 8: Schematic diagram of the force recording.

AC Force

Under normal conditions, force produced by the contractile machinery is observed to have a certain amount of fluctuations. To study the extent of changes with respect to prolonged firing of the motoneuron, force fluctuations were monitored. These force fluctuations were obtained by filtering the DC force from the bridge amplifier with a bandpass of 1 - 50 Hz. The resultant AC force signal was monitored throughout the experiment in the oscilloscope. The traces of the AC force were acquired on the computer (100 Hz sampling rate) and stored on a videotape for off-line analysis.

Data Acquisition

Data analysis was done using SPIKE2 software from Cambridge Electronics Division (Cambridge, U.K.). The shape of each single motor unit spike was discriminated using Bak (U.S.A) time-voltage window discriminators. Each spike was thus converted to a transistor-transistor-logic (TTL) pulse. Data were acquired into the computer using the following acquisition rates for various recordings shown in Fig. 9:

Single motor units -13 KHz

TTL-100 Hz

EMG-6 KHz

DC Force-50 Hz

AC Force-100 Hz

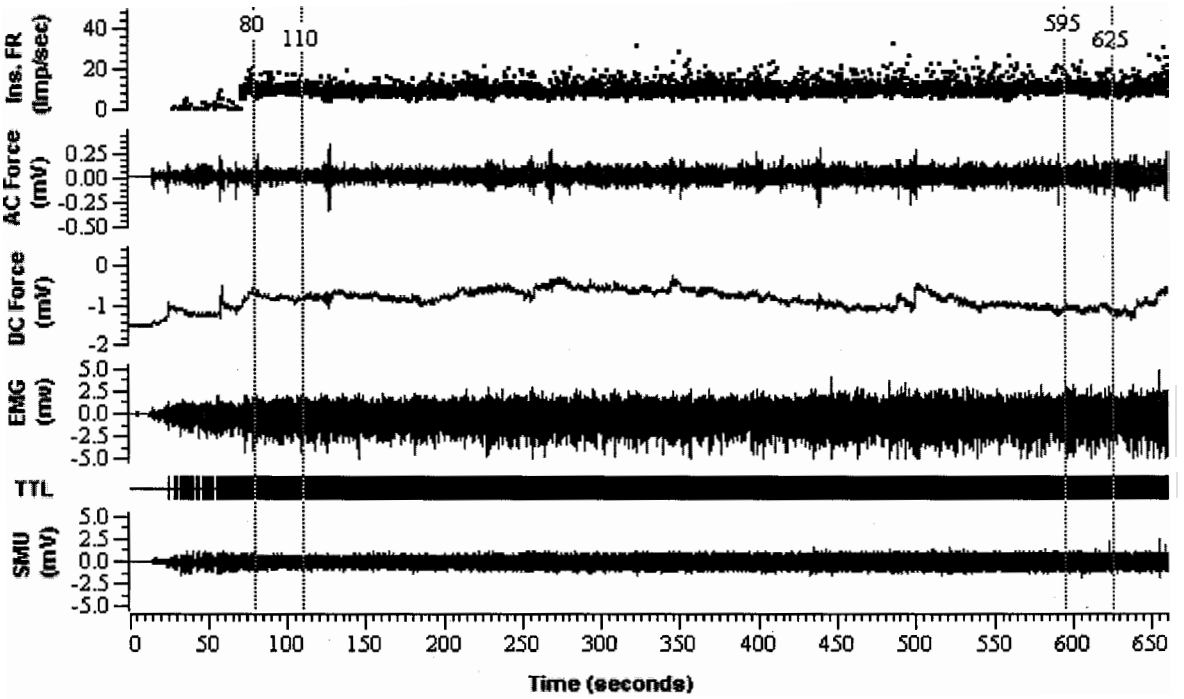

Figure 9: Sample record of all channels. SMU is the raw motor unit data, TTL channel contains the TTL pulses for the discriminated unit, EMG channel shows the time course of unrectified surface EMG. DC force represents the force of abduction and AC record is the filtered force. Instantaneous firing rate shown in the top panel was obtained from TTL pulses in channel 2. The two pairs of cursors indicate duration of 30 seconds; data between the cursors was used for various analyses.

After data acquisition, motor unit data (bottom trace on fig. 10) were corrected for any wrong discrimination using a special script. The TTL data (middle trace on fig.10) were then converted to instantaneous firing rate as shown in the top trace on Fig. 10. The specific analysis for each parameter is described in the following paragraphs.

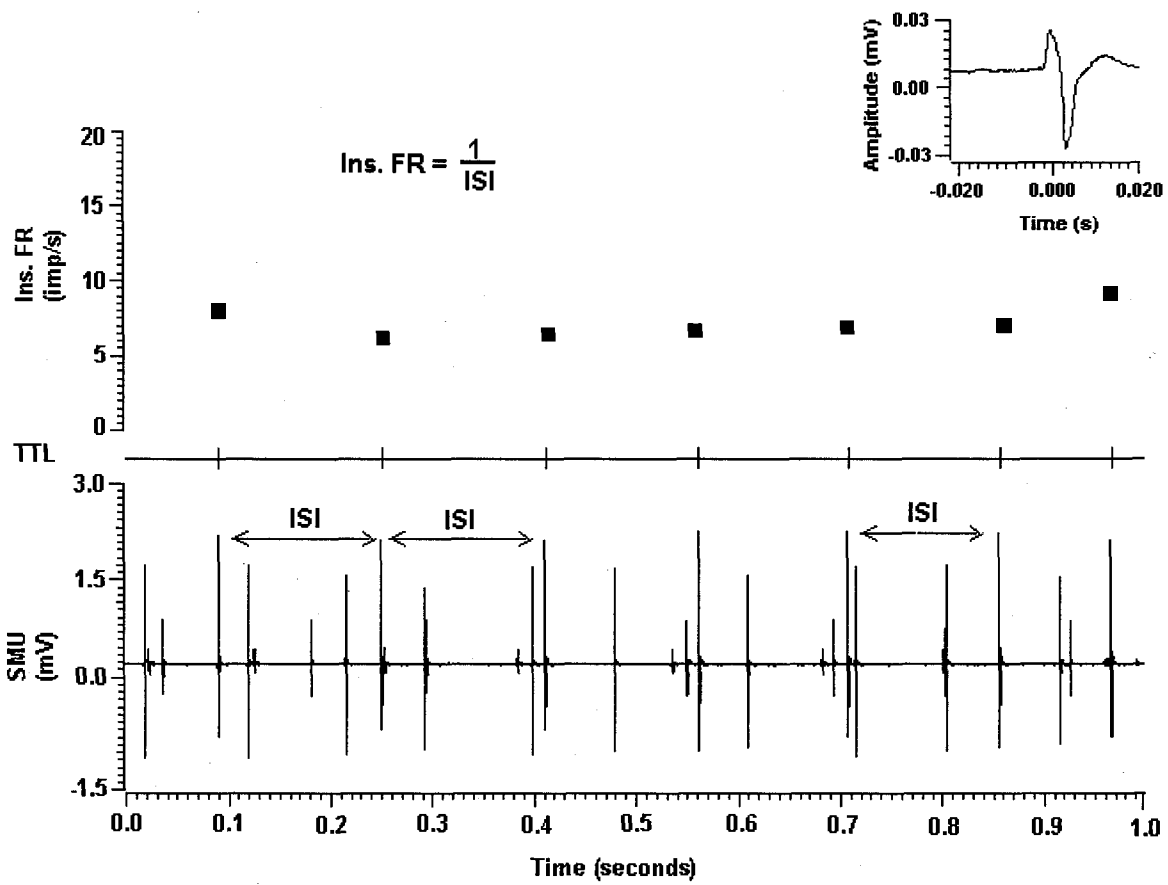

Figure 10: Sample record of the SMU (bottom trace), TTL (middle trace) and Instantaneous firing rate (top trace) for a one second duration. The motor unit action potential of the unit that is being monitored is shown on the top left-hand side.

Data Analysis

After raw data were acquired on the computer, various physiological records were used to compute changes in properties of the motor unit after it had discharged for a prolonged period. Computations were done to assess changes in threshold of

motoneuron, inter-spike interval, changes at the neuromuscular junction, and changes in the magnitude of fluctuations in force.

The first step was to identify the periods of constant firing at the beginning and at the end of the long run. This was first done by visual inspection of the instantaneous firing rate record following which 30 or 60 seconds of data were selected to compute firing rate during the initial and the final periods. For 58 of the 60 units, firing rate changed by less than 10 % from the initial to the final period, while for the other two, it changed by more than 10 % between the in initial and the final parts of the run. See under "Statistics" the method to determine the duration of data (30 or 60 s) chosen to compare initial and final values of a particular parameter.

EMG magnitude

For computing changes in the magnitude of EMG, the EMG was rectified and mean values of EMG for the initial and final 30 s were compared. The significance of change was assessed by dependent t-test for the population of 58 motor units.

Statistical properties of the motoneuron

The statistical properties of the motoneuron firing were determined by computing the first order interval histograms for the initial and the final 30-s of constant firing rate periods. From the histograms, means and standard deviations were obtained using the distribution of inter-spike intervals. For each run coefficient of variation of ISI was calculated from the mean and standard deviation (coefficient of variation = standard

deviation / mean). The initial and the final values of coefficient of variation were compared by dependent t-test to assess changes over time for all 60 motor units.

Changes at the neuromuscular junction and sarcolemma

Changes at the neuromuscular junction (NMJ) and sarcolemma were studied by looking at changes in two different parameters: a) motor unit action potential and b) median power frequency.

a) Motor unit action potential

The needle electrode, that is used to record single motor units, samples very few muscle fibres of the whole motor unit. Therefore the recorded spike is of short duration and does not give information about the total duration of the motor unit action potential (Stalberg, et al., 1996). Using the TTL output of the recorded spike as a trigger, an estimate of the motor unit action potential representing the whole motor unit was obtained from the EMG recording using the spike triggered averaging technique. The computed MUAP is only an estimate since all muscle fibres of the motor unit do not contribute equally to surface recorded EMG. A sample of such an average is shown in Fig. 11 with the recorded spike on the top and the spike-triggered-averaged motor unit potential (MUAP) in the lower panel.

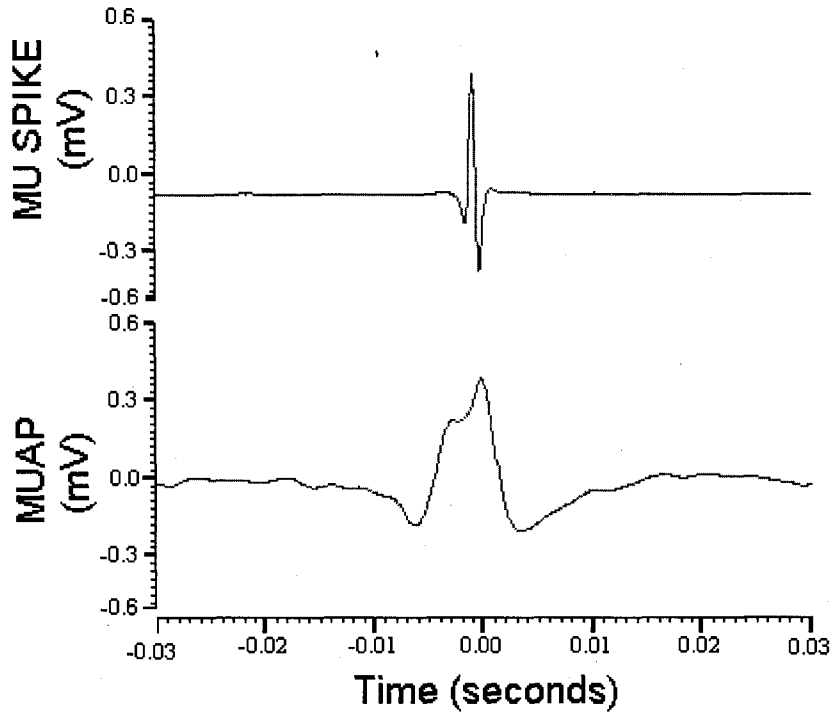

Figure 11: The two traces refer to the MU spike (upper trace) recorded by the intramuscular microelectrode and the MUAP (lower trace) obtained from the EMG using the spike triggered averaging technique.

The peak to peak duration of the action potential was measured by placing two vertical cursors in line with the peaks identified in the motor unit action potential. A sample measurement of the motor unit action potential is shown on Fig. 12. Changes between the initial and final duration of the motor unit action potential was then tested using a paired t-test. The amplitude of MUAP was measured between the positive and negative peaks of MUAP by placing two horizontal cursors as shown on Fig. 12.

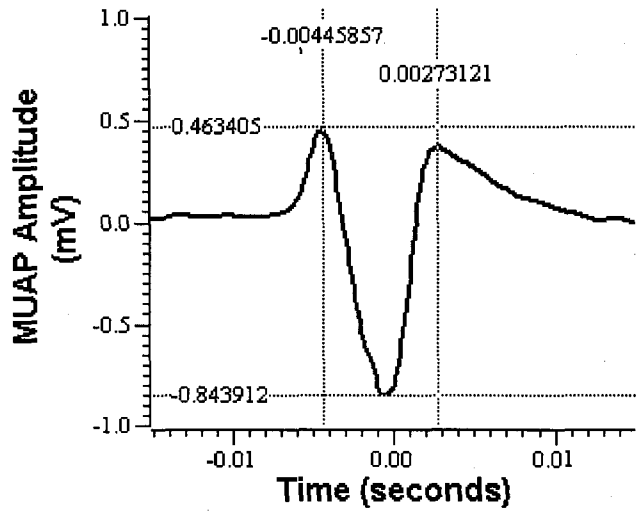

Figure 12: Sample figure showing the measurement of amplitude and duration of MUAP. The duration is measured by measuring the distance between the two vertical cursors. The amplitude is measured by measuring the distance between the two horizontal cursors.

b) Median Power Frequency

In this study, the changes in the EMG power spectrum were analysed by determining the median power frequency from the EMG power spectrum. A thirty second data sample was selected for both the initial and final periods of a run for analysis. Data from these regions were fast Fourier transformed, and the resultant power spectrum was computed (Fig. 13). The resultant power spectrum was analysed by placing cursors at 5 and 2000 Hz. These frequencies were chosen as not much of activity was observed beyond these two regions. Once this was done the distal cursor was brought towards the centre until it reached half of total power, and the corresponding frequency as identified from the X-axis, was taken as the median power frequency.

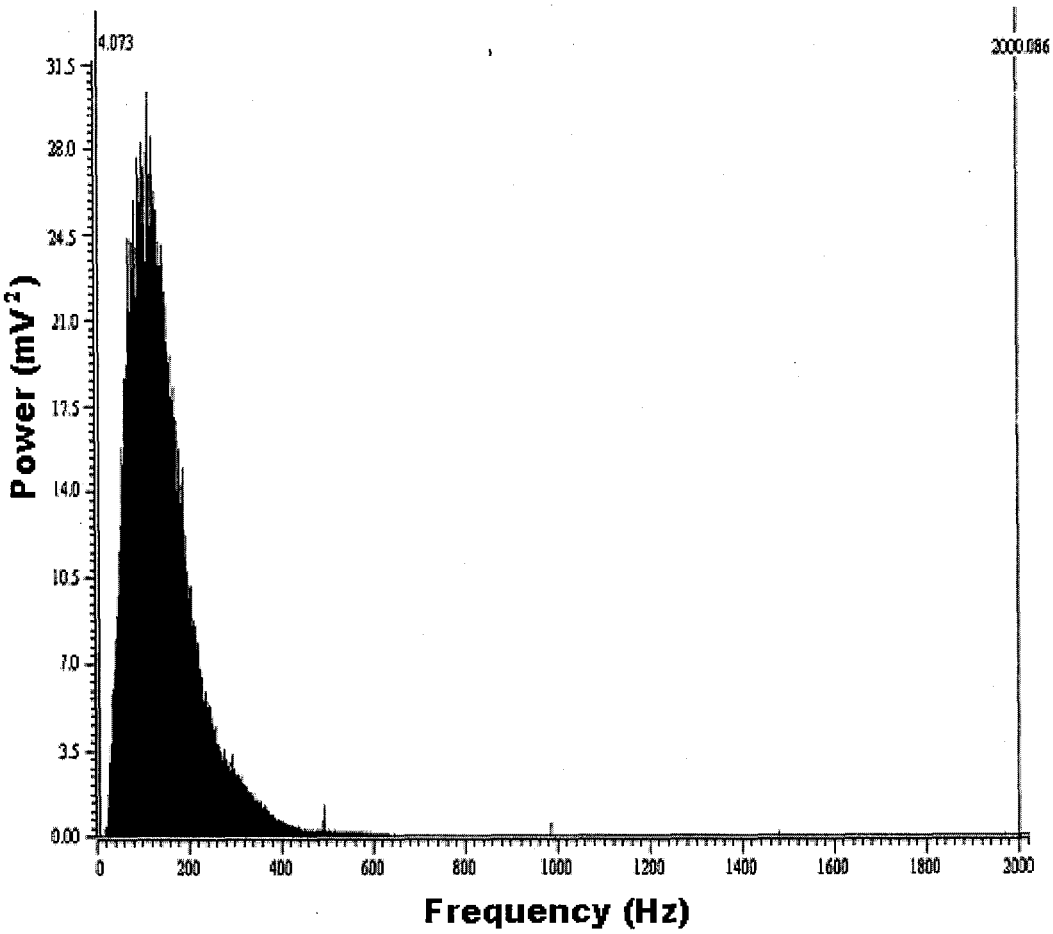

Figure 13: Sample power spectrum record. Cursors were placed at 5 and 2000 Hz of the spectrum and the area between the cursors was used to determine the median power frequency. (All values in the Y-axis are multiples of 10^{-6}).

Changes in the magnitude of force fluctuations

For analysis of changes in the magnitude of AC force fluctuations, the standard deviation of the amplitude of AC force was computed for initial and final 30 s of AC force record. These two values of SD were compared for change over time using paired t-test.

Relationship between 'targeted' and 'non-targeted' units

As mentioned earlier, along with the targeted motor units, additional clear units were recorded frequently during some experiments; we called these as the non-targeted units. Some of the non-targeted motor units fired from the onset of the run with targeted units while the others were recruited later during the discharge of the targeted unit. Each of the discernible non-targeted motor unit was discriminated off-line and acquired on the computer using 13 KHz for the raw SMU record and 100 Hz for the corresponding TTL pulses. The discrimination of TTL pulses were corrected for any mistakes in discrimination. The TTL pulse train was converted to instantaneous firing rate on SPIKE2.

Since the firing rate of the targeted motoneuron was kept constant in this study, we wanted to examine if the non-targeted units changed their firing characteristics while the targeted motor unit maintained its rate. To check for constancy of firing rates, we compared slopes of firing rates of targeted and non-targeted units during the periods both firing. In addition, we tested for changes in coefficient of variation of the non-targeted units.

Statistics

The first step in analysis was to pick a small sample of data that would reliably represent the initial value of the parameter (the same for the final value). For this purpose intra-class reliability test was done using SPSS statistical package.

EMG magnitude

The mean EMG activity was computed by selecting two consecutive 15 s periods of rectified EMG from the initial and final stages of each run. To determine the duration of initial and final sample, two consecutive 15 s periods of data were selected. Single measure intra-class correlation (SMIC) was conducted for the two 15 s measures. In addition, the average measure intra-class correlation (AMIC) was computed for the mean values of the two 15 s values. Since SMIC was not always high, we used AMIC, effectively using 30 s of data from the initial and the final periods of a run to compare the two values.

For mean EMG magnitude from the initial periods of the run had a SMIC = 0.9924 and an AMIC = 0.9962, and for the final periods SMIC = 0.9948 and AMIC = 0.9974. AMIC values were used for final analyses. To test for significant changes from initial to final values a t-test for correlated means was performed.

Changes in the statistical properties of the motoneuron

The means and standard deviation of inter-spike intervals was computed from the first order interval histograms. Mean ISI had an SMIC = 0.9692, and AMIC = 0.9844 for the initial two consecutive15 s and SMIC = 0.9764 and AMIC = 0.9929 for the final two 15 s periods. For SD, SMIC = 0.7966, and AMIC = 0.8868 for the initial two 15 s samples and SMIC = 0.9099 and AMIC = 0.9528 for the final two 15 s samples of ISI data. As mentioned before the AMIC was used for analysis of data. For ISI data analysis, AMIC was used for both means and SD, and the coefficient of variation was calculated from the average of two initial and two final measurements. To determine if

any changes had occurred between the initial and final coefficient of variation, t-test for correlated means was performed.

Motor unit action potential

MUAPs were computed for two consecutive 60 s initial periods. In order to assess changes in the duration of motor unit action potential, we had to use a 60 s sample as determined by intra-class reliability test.

For MUAP computed from the initial two consecutive 60 s data, we obtained SMIC = 0.9572 and AMIC = 0.9782. We used SMIC due to short lengths of certain runs. No reliability test was performed for the final values due to the same reason. But we assumed that the values of SMIC would not be too far off from the ones obtained for the initial samples. Statistical changes between the initial and final stages were assessed using a t-test for correlated means of initial and final MUAPs.

Median power frequency

Power spectra were computed from two consecutive periods of unrectified EMG samples of 15 s duration each, from both the initial and final stages of a run. Median power frequency (MPF) was determined for each spectrum. Reliability test of these MPF values was performed. For the initial part of the data for MPF, SMIC = 0.9809 and AMIC = 0.9903 were obtained, and for the final EMG, MPF had SMIC = 0.9878 and AMIC = 0.9939. To assess significance of changes between the mean values of MPF from the initial to the final values of EMG, a correlated t-test was done.

AC Force

Variability over time in the force fluctuations was quantified by computing the standard deviation of force over two 15-second duration periods from both the initial and final stages of a run. Subsequently, the two consecutive periods of data of 15-s duration each, from both initial and final stages of a run was subjected to a reliability analysis. For the initial SD of AC force fluctuations, SMIC = 0.9798 and AMIC = 0.9898, while for the final samples SMIC = 0.9573 and AMIC = 0.9782. Statistical changes for the force fluctuations between the initial and final stages were assessed using a t-test for correlated means.

Targeted and non-targeted units

Two tests were used for studying non-targeted units. First, the slopes of firing rate versus time for the targeted and non-targeted units were compared using independent t-test. As for the targeted motor units, coefficient of variation was calculated for the initial and the final 30 s of firing of each non-targeted motor unit. Comparisons between the initial and the final coefficient of variation were made using dependent t-test.

Differences between fast and slow units

All recorded motor units were divided into two groups based on the mean inter-spike interval. All motor units with ISI greater than 100 ms were classified as 'slow motor units'; motor units with ISI less than 100 ms were classified as 'fast motor units'. This classification enabled us to examine if the magnitude of change in any parameter depended on the firing rate between motor units firing at different firing rates. Mean

percent changes in coefficient of variation, EMG amplitude, MUAP, MPF and standard deviation of AC force were compared between the two groups. Significance of the magnitude of changes between the two groups was assessed using independent t-test for two-samples with unequal variances.

RESULTS

Data are reported from 60 targeted and 16 non-targeted single motor units. Data were analyzed to find any possible changes in mean EMG magnitude, statistical properties of the motoneuron, median power frequency (MPF) of EMG power spectrum, the duration of the motor unit action potential, and the amplitude of fluctuations in force when these motor units discharged for prolonged periods. These 60 units were divided into two groups; fast and slow. The magnitude of changes were compared for the fast (ISI < 100 ms) with the slow motor units (ISI > 100 ms). The slope of instantaneous firing rate versus time of a non-targeted unit was compared to that of the corresponding targeted unit to examine the existence of parallel behavior between the two.

The duration of motor unit firing, during which the unit fired at a constant rate, differed between the various motor units. Some motor units held constant rate for longer than 10 minutes while some fired for less than 3 minutes. These differences in duration depended on factors including recruitment threshold, firing rate of the motoneuron and also on the ability to isolate the targeted unit online. All units that were studied were recorded either until the subject was unable to maintain the firing rate or when the discrimination of the targeted motor unit became difficult due to the interference from the additional motor units. The information on mean ISI and duration of firing of each motor unit are presented in appendix B.

The subject was asked to recruit a motor unit that could be discriminated. Once recruited, he was asked to increase the firing rate and then hold that new rate for approximately 10 minutes. This duration of the period between when the unit started to

discharge and when the beginning of the constant rate was reached (d in Fig. 14) ranged from 3 to 75 seconds, with a mean duration of 22.73 ± 17.50 seconds. Once a constant firing rate was achieved, the motor unit was recorded until it ceased to fire. This duration of constant firing rate is marked as 'X' in Fig. 14. The duration of X ranged from 73 to 1140 seconds, with a mean of 448.25 ± 226.65 seconds.

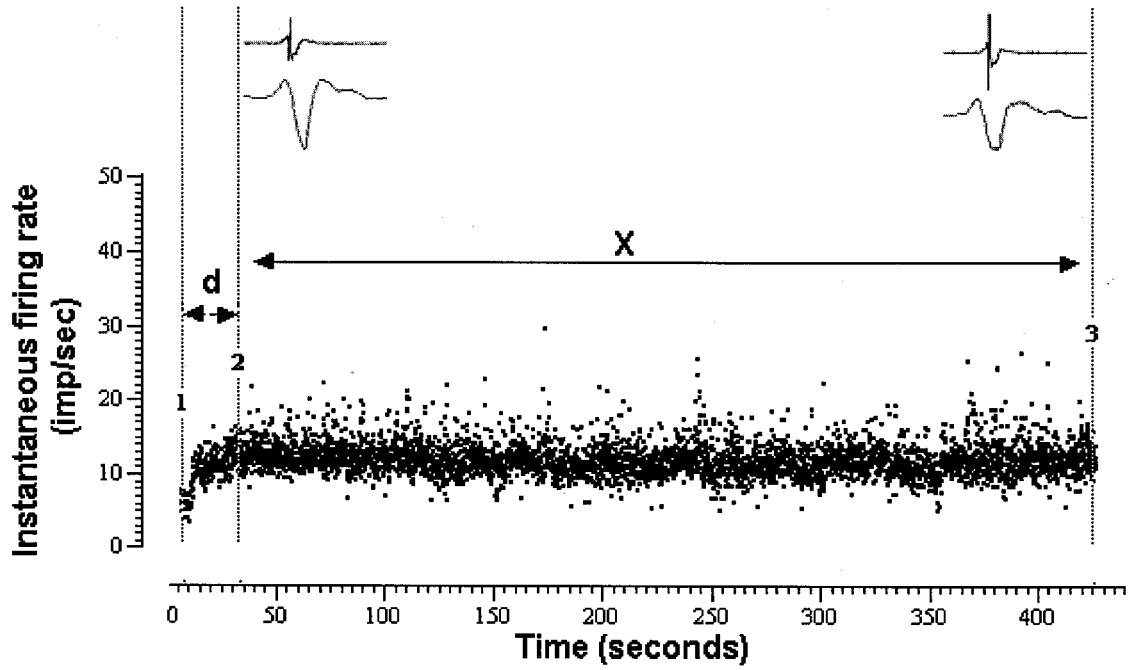

Figure 14; the figure above represents the firing pattern of a motor unit. Region "d" between cursor 1 and cursor 2 is the time taken by the motor unit to achieve constant firing rate. Region between the cursors 2 and 3 ('X') is the total duration the motor unit fired with a constant firing rate. On the top are shown action potentials averaged over 30 s of data during the initial and the final periods of constant firing rate. The narrow top action potential represents the activity recorded by the microelectrode while the action potential below that was obtained by spike triggered averaging of surface EMG with TTL of the discriminated unit. This wider potential is a better representation of the whole motor unit action potential (MUAP).

Among the motor units that were studied, the firing rates averaged from approximately 7 imp·s^{-1} to 15 imp·s^{-1}. The ISI of the 60 targeted motor units ranged from 66.7 ms to 194.1 ms. Based on the ISI, motor units were classified as fast and slow motor units. Units that fired with ISI greater than 100 ms were termed as 'slow units' and units that fired with ISI lesser than 100 ms were termed as 'fast units'. The slow units fired

with ISI ranging from 103.3 to 194.1 ms (mean ± SD = 125.95 ± 24.01), while the ISI for fast units ranged from 66.7 to 99.4 ms (mean ± SD = 82.13 ± 8.30). Total firing duration of the slow motor units from 160 seconds to 1140 seconds, with a mean duration of 554.09 ± 229.953, while the fast units had duration ranging from 73 seconds to 675 seconds, with a mean of 382.46 ± 200.68 seconds. The distribution of ISI and their corresponding duration of constant firing rate are shown in Fig. 15. The greater the ISI, the longer was the duration for which the subject could maintain constant firing. The difference between the mean duration of the two groups was significant at the level of $p = 0.003$ (independent t-test).

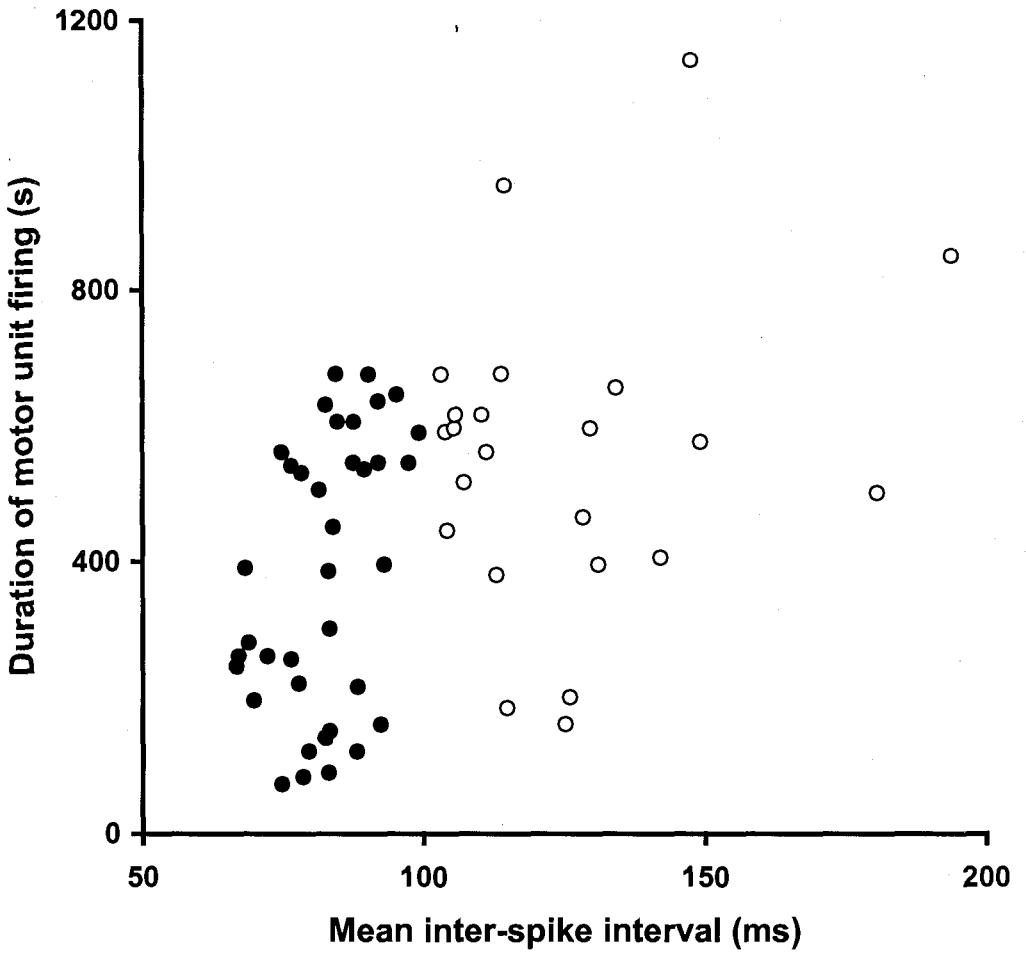

Figure 15: ISI of all motor units is plotted along the abscissa and the corresponding duration of constant firing rate along the ordinate. Slow units (ISI > 100 ms) are represented by open circles, fast units (ISI < 100 ms) are shown by filled circles.

For these experiments, it was very important for subjects to maintain a constant firing rate, but it was not always possible. The percent change in ISI between the initial and the final sample periods were identified for analysis. Two of the 60 units stood out clearly for percentage change in ISI (percent ΔISI) greater than 10 %; changes in the rest of 58 units were less than 10 %. Those two units were omitted from analysis for changes in EMG magnitude. For MUAP analysis, only 56 of the 60 motor units were used since the intraclass reliability test dictated use of 60 s of data for the initial and final sample

periods and for four of the 60 units this was not possible. For analysis of force fluctuations, only for 29 units were used for final statistics because calibrated force data were not available for the rest.

In terms of EMG, the maximal voluntary contraction for the five subjects ranged from 0.75 mV to 1.18 mV. The magnitude of EMG during the constant firing rate ranged from 0.2 % to 17.5 % MVC with a mean of 5.4 ± 4.2 % MVC for the initial period, and from 0.3 % to 22.3 % MVC with mean of 6.8 ± 5.5 % MVC for the final period.

Changes in electromyographic activity

Constant force paradigms have shown that, in general, a decline in firing rate occurs while force is kept constant. Does the motoneuron fatigue? If it does, it would need additional input to maintain a constant firing rate. This additional input must be evident with increased command signals to the motoneuron pool, which must be reflected in the magnitude of EMG activity. To investigate this comparisons were made between the initial and final magnitude of mean EMG for 58 units during constant rate periods. The final mean EMG magnitude (final mean ± SD = 0.0585 ± 0.0455 mV) was significantly higher than the initial mean EMG magnitude (initial mean ± SD = 0.0465 ± 0.0360 mV) (paired t-test, $t_{58} = 5.430$, $p < 0.001$). The relationship between the initial and final mean EMG magnitude is presented on Fig. 16 for 58 motor units (these data are tabulated in Appendix C).

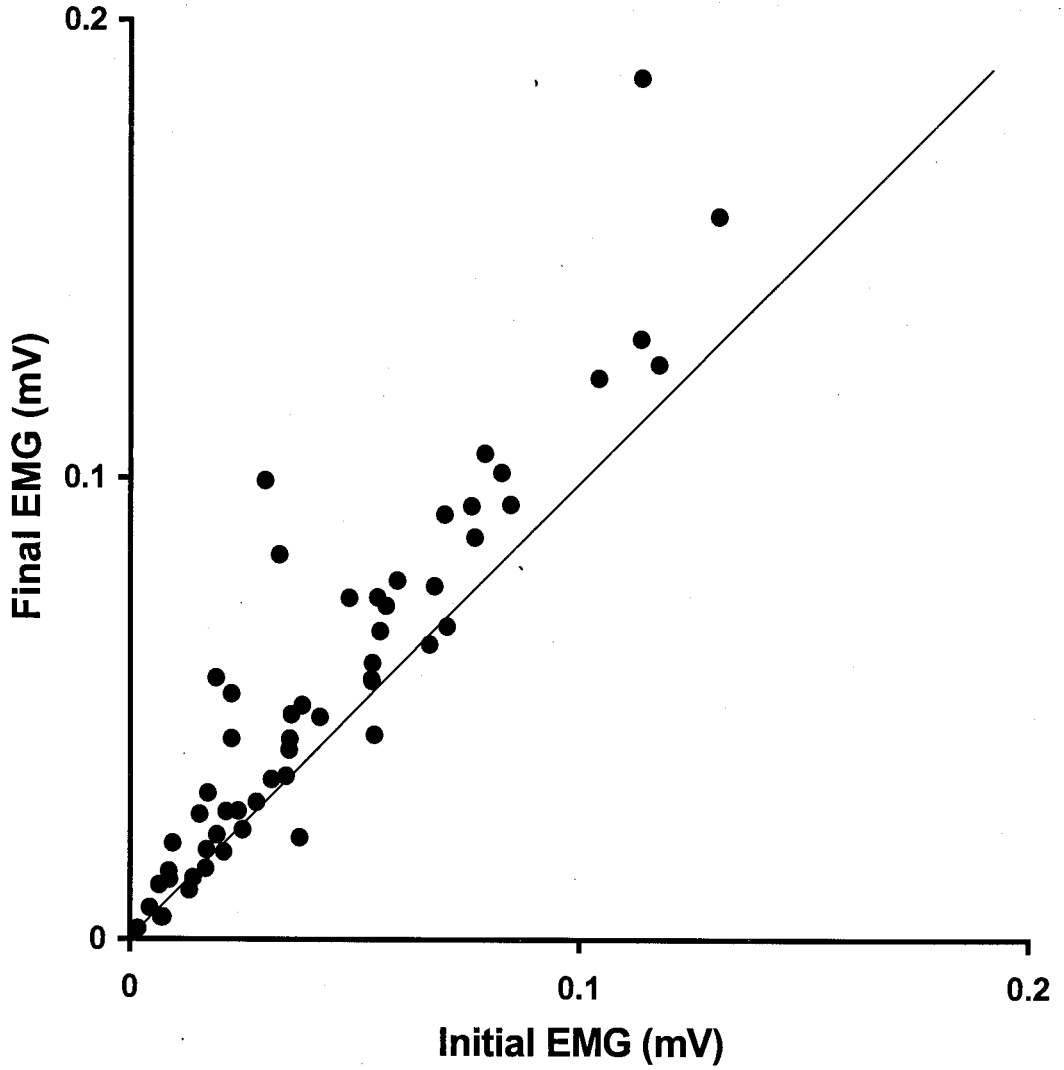

Figure 16: Changes in the mean EMG magnitude from the initial 30 s to the final 30 s of data. The initial mean EMG magnitude values are represented along the abscissa, and the final mean EMG magnitude values along the ordinate. Points lying above the line of identity indicate an increase in the EMG magnitude.

The figure above shows that, in general, EMG magnitude during the final period was higher compared to initial corresponding value. To investigate if EMG magnitude changes were dependent on the motoneuron firing rate, the percentage change in EMG was plotted against the respective ISI for fast and slow motor units (Fig. 17). The fast motor units had a change in EMG magnitude of 41.85 ± 55.38 %, while the slow motor units had a change of 19.19 ± 41.62 %. This difference between fast and slow motor units

was statistically significant (independent t-test with unequal variance, $t_{55} = 1.673$, $p = 0.041$).

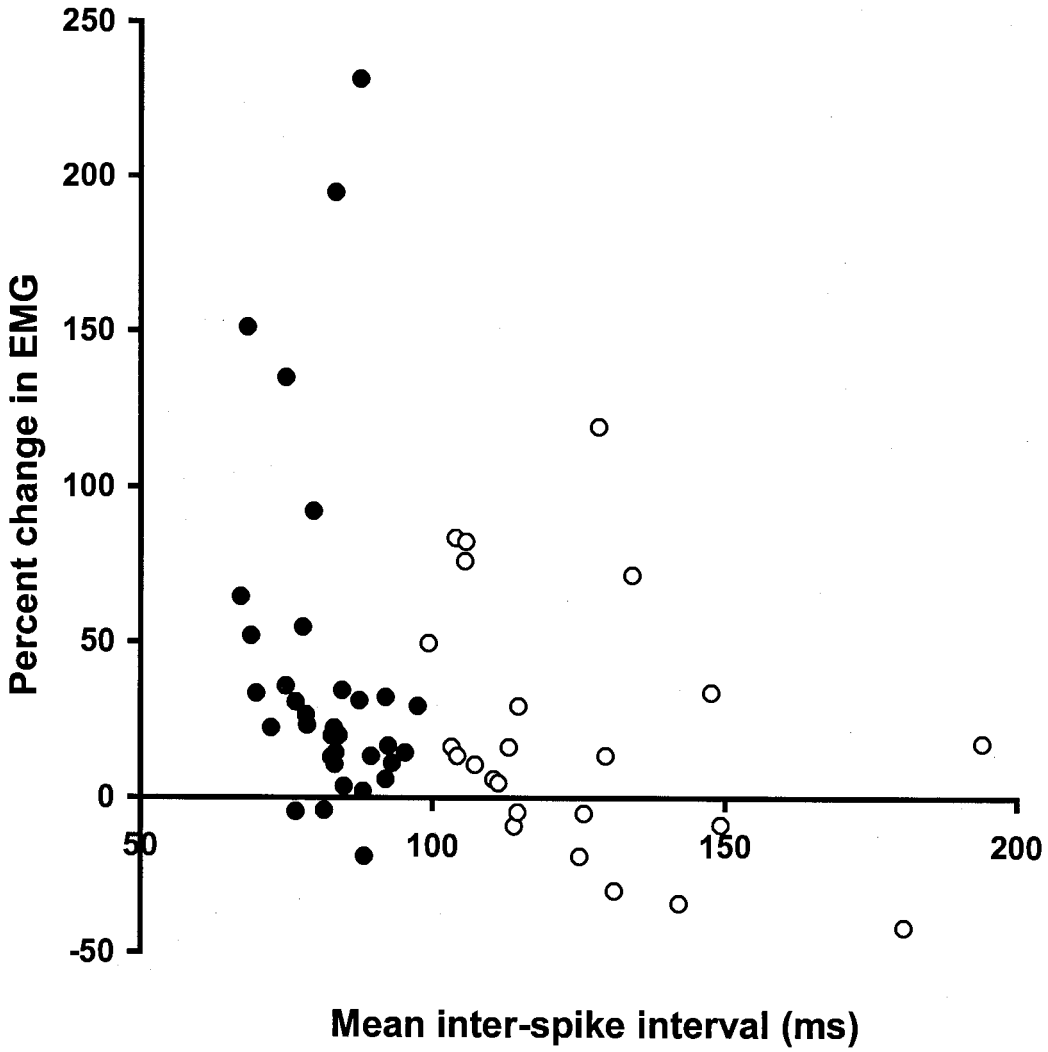

Figure 17: ISIs of the motoneurons are plotted along the abcissa, and percent change in EMG along the ordinate. Closed circles present fast motor units, while open circles present slow motor units.

Statistical properties of the motoneuron

Research in the past examined changes in statistical properties of a motoneuron using a constant force paradigm. Nordstrom and Miles (1991a,b), examined changes in coefficient of variation using constant rate paradigm in masseter muscle which is innervated by brainstem motoneurons. No such information is available on motoneuron properties when the subject is asked to maintain firing rate constant for limb muscles innervated by spinal motoneurons. We were interested to see what would happen to the coefficient of variation if the subject maintained a constant firing rate using the first dorsal interosseous muscle.

For this purpose first order interval histograms were computed for the initial and final 30 seconds of the data (for all 60 motor units) and the means and standard deviation were obtained from the distribution of ISIs. The coefficient of variation was calculated from the corresponding mean and standard deviation. Analysis revealed that the coefficient of variation increased from an initial mean value of 0.182 ± 0.072 to the final value of $0.234 \pm .096$ and this difference was statistically significant (paired t-test, $t_{59} = -5.981$, $p < 0.001$). This observed effect between the initial and final coefficient of variation for all 60 units is plotted on Fig. 18 with the initial coefficient of variation along the abscissca and the final coefficient of variation along the ordinate (detailed data are tabulated in Appendix D).

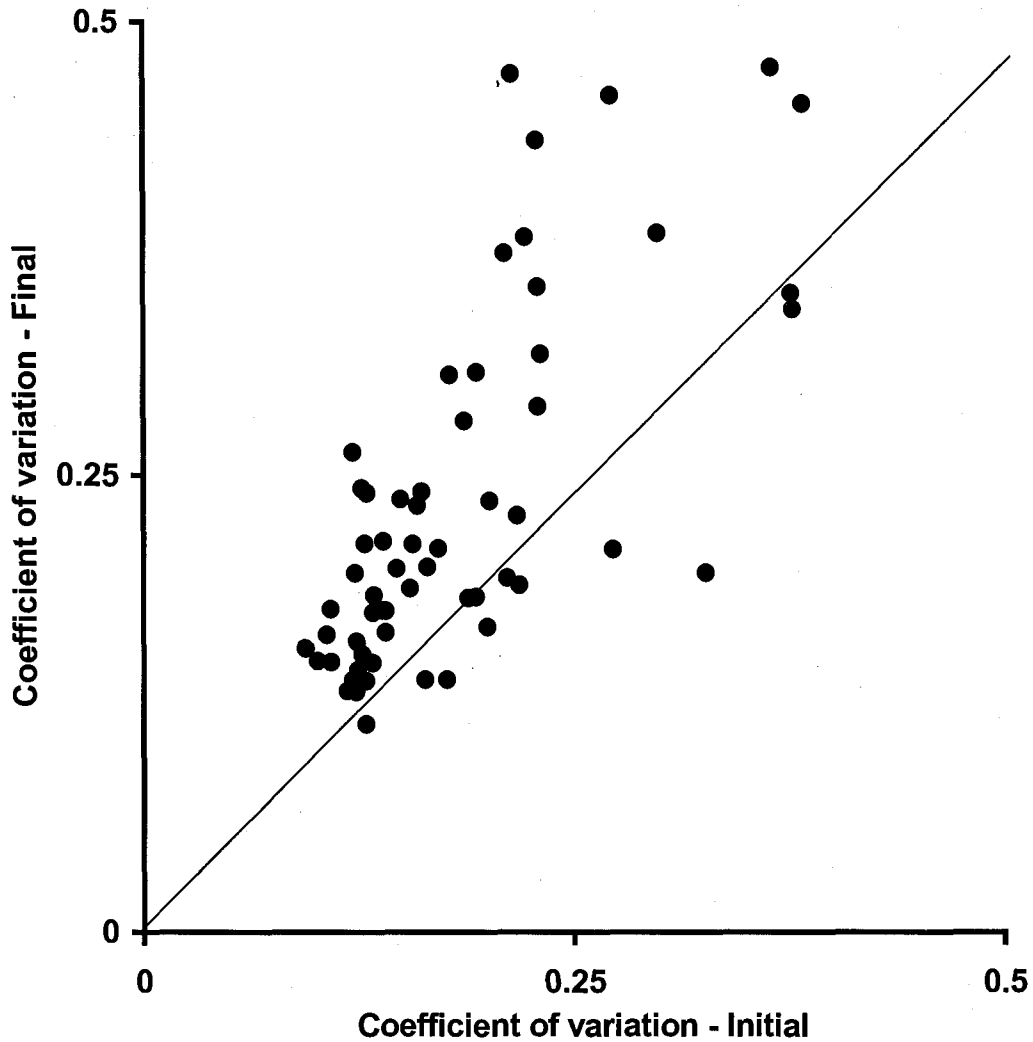

Figure 18: This figure illustrates the changes that occurred in the coefficient of variation in ISI as a result of prolonged firing. The initial coefficient of variation is plotted along the abscissa and the final corresponding values are along the ordinate.

Further analysis was done to see if these changes were the same for the fast (ISI < 100 ms) and slow (ISI >100 ms) motor units. The percent change in coefficient of variation was 31.65 ± 32.50 for the fast motor units (N = 37), and 31.92 ± 38.56 for the slow motor units (N = 23). The difference between the fast and the slow motor units were not statistically significant (independent t-test, $t_{41} = 1.683$, $p = 0.49$). The relationship between the fast and the slow units for the whole population is presented on Fig. 19.

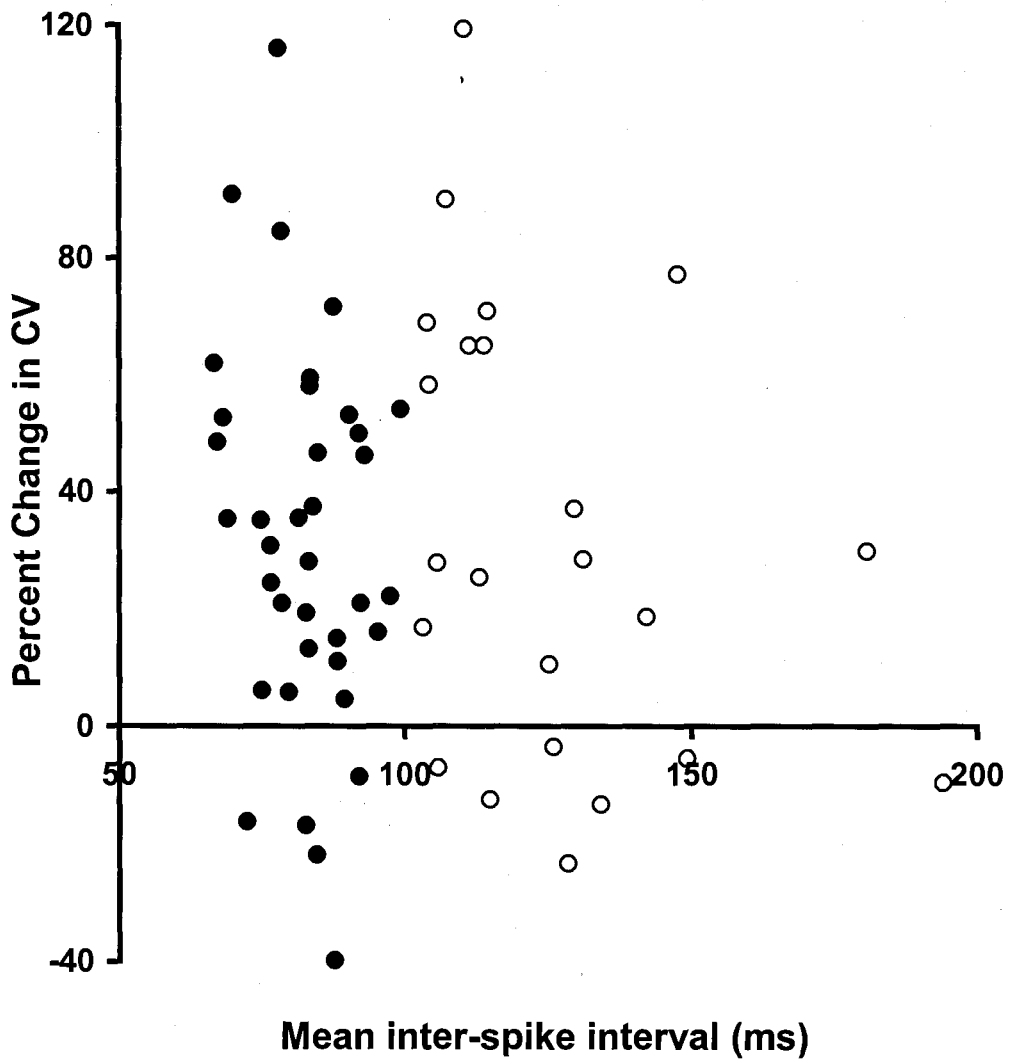

Figure 19: All motor units are plotted with their ISI on the abscissa and their respective percentage change in coefficient of variation on the ordinate. Open circles represent slow units and solid circles represent fast units.

Neuromuscular junction and the sarcolemma

The action potential of a motoneuron travels from the axon hillock to the motoneuron terminal and then to the effector muscle unit. During the prolonged firing of the motoneuron, if any changes occur in the motoneuron that cause a change in conduction velocity and/or neuromuscular transmission failure at the junction, then such changes must be reflected in the motor unit action potential and median power frequency

of EMG. We assessed the changes by examining the profiles of motor unit action potentials and power spectrum of EMG.

a) Changes in Motor unit action potential

The duration of an action potential would be expected to stay the same if there were no change in axon terminals, release of neurotransmitter or conduction of action potential along the sarcolemma. If axon terminals underwent hyperpolarization, and/or changes in sarcolemma occurred, these changes would be reflected as an increase in action potential duration. To investigate such changes, motor unit action potential (MUAP) was estimated by spike-triggered averaging of unrectified surface EMG, using TTL as trigger. The comparison revealed a significant increase in action potential duration from 7.7 ± 1.8 ms (initial MUAP) to 8.5 ± 2.4 ms (final MUAP). This increase was statistically significant (paired t-test, $t_{55} = -5.135$, $p < 0.001$). The relationship between initial and the final MUAPs is presented on Fig. 20 for 56 units and all numbers are tabulated in Appendix E.

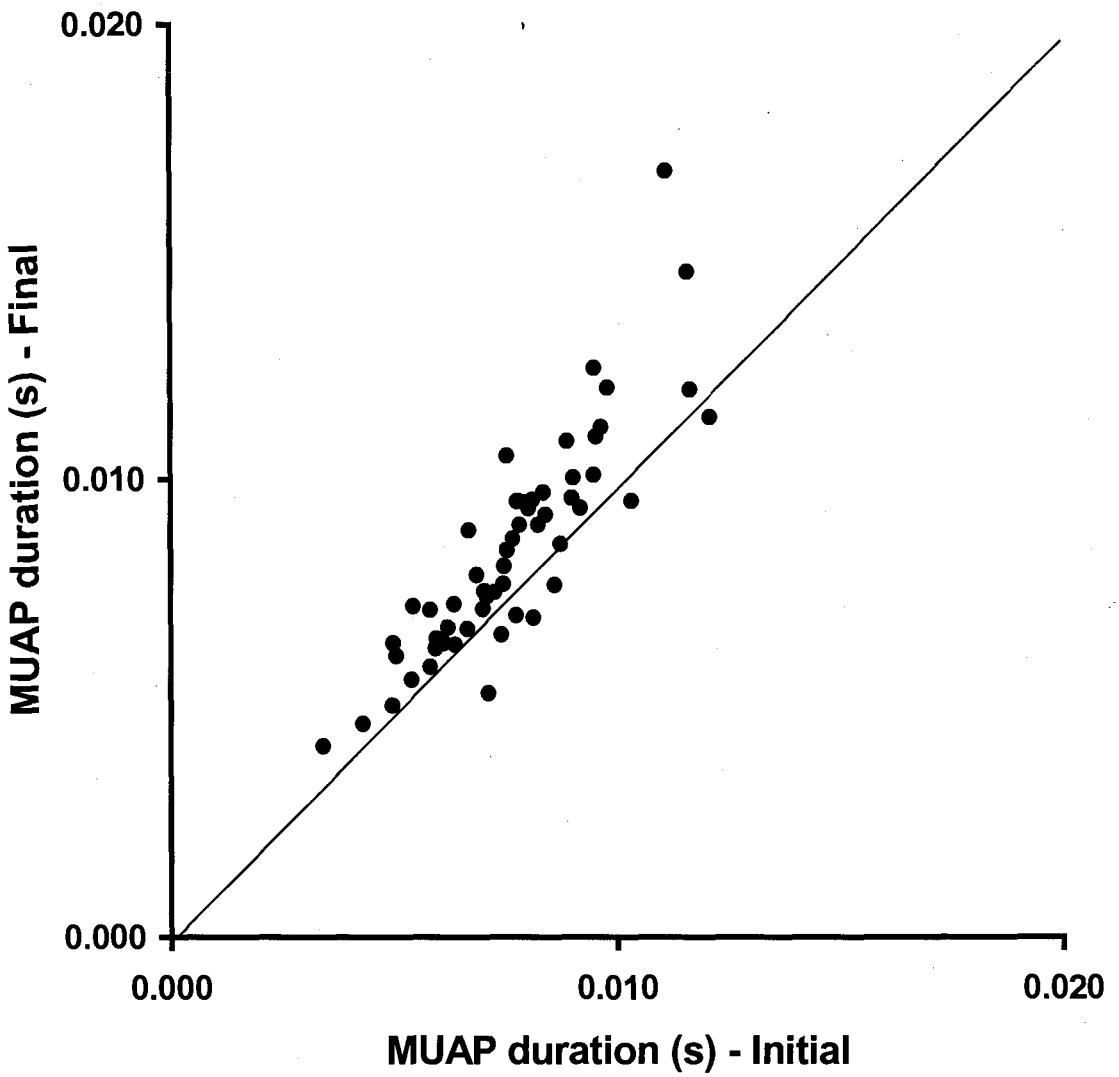

Figure 20: Changes in the motor unit action potential. The initial duration of the action potential is plotted on the abscissa, with the corresponding final action potential duration is plotted on the ordinate.

To see if these changes were different for fast motor units when compared to those in slow motor units, comparisons were made between magnitude of change between the two sets of motor units. MUAP of fast motor units increased in duration by 10.67 ± 13.77 %, while MUAP of slow motor units increased by 10.70 ± 14.12 %. The difference between the fast and slow motor units was not statistically significant

(independent t-test with unequal variances, $t_{47} = 1.678$, $p = 0.496$), indicating very little relationship between change action potential duration and firing rate. These data are presented in Fig. 21.

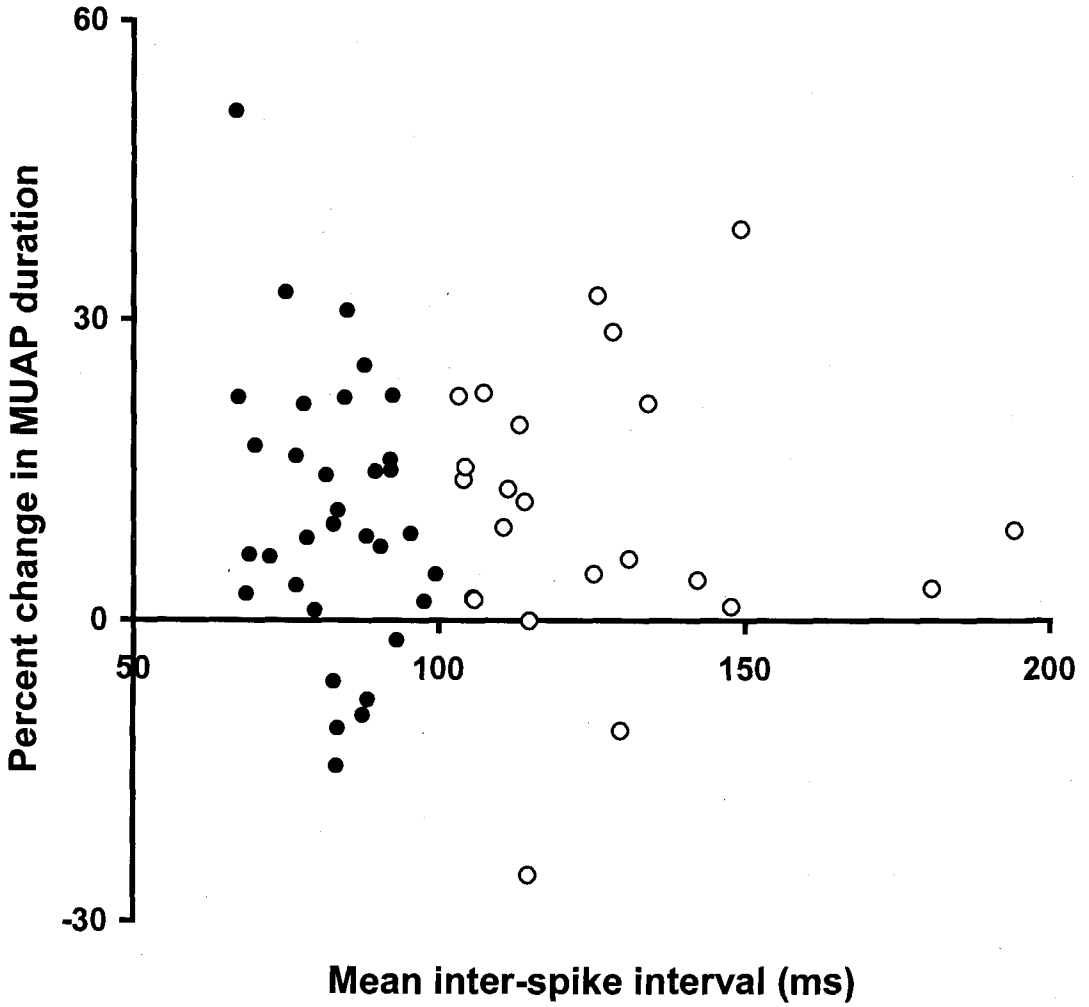

Figure 21: This figure represents the changes in the magnitude of MUAP duration. The ISI of the motor units is presented along the abscissa, with the percent change in MUAP duration along the ordinate. Closed circles represent fast motor units, while open circles represent slow motor units.

Analyses were done to observe changes in the MUAP amplitude. The mean final MUAP amplitude (mean ± SD = 0.1572 ± 0.1565) was higher than the initial MUAP amplitude (mean ± SD = 0.1499 ± 0.1404). This difference was not statistically different

(paired t –test, $t_{56} = -1.616$, $p = 0.056$). The relationship between the two is presented in Fig. 22, and detailed data is tabulated in Appendix F.

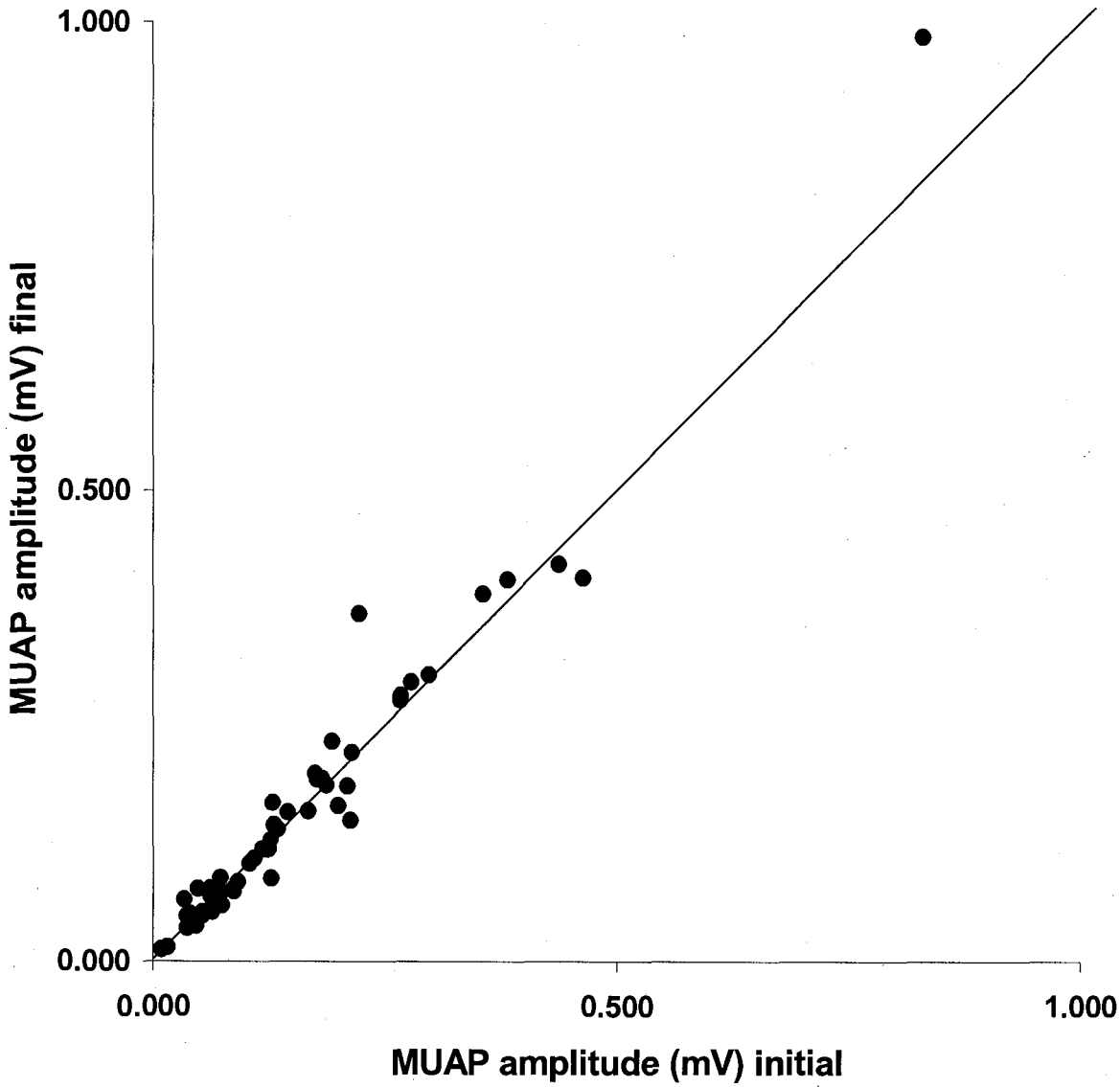

Figure 22: Changes in the amplitude of the motor unit action potential. The initial amplitude of the action potential is plotted on the abscissa, with the final action potential amplitude plotted on the ordinate.

To see if there were any changes in MUAP amplitude between fast and slow motor units, comparisons were made between the magnitude of change for the two sets of motor units. MUAP amplitude for fast motor units increased with a mean of 8.68 ± 26.94

%, while slow motor units had a mean increase of 3.02 ± 11.43 %. Though the mean increase for the fast was higher than the slow units, the difference between the two was not statistically significant (independent t-test with unequal variances, $t_{45} = 1.679$, $p = 0.109$), indicating very little relationship between the change in amplitude of the action potential and the firing rate. The relationship between the firing rate and the change in amplitude of the action potential is presented in Fig. 23.

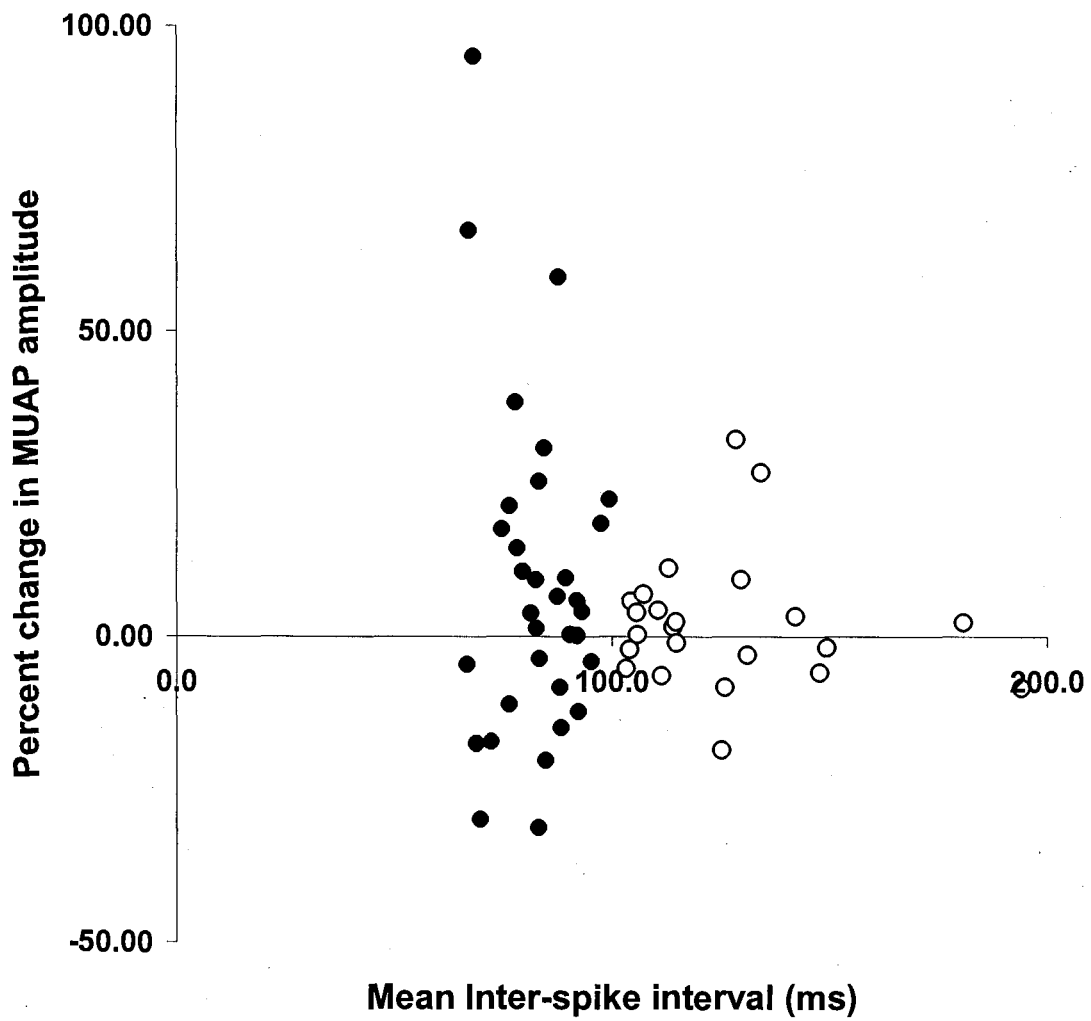

Figure 23: This figure represents the changes in the magnitude of MUAP amplitude. The ISI of the motor units is presented along the abscissa, with the percent change in MUAP amplitude along the ordinate. Closed circles represent fast motor units, while open circles represent slow motor units.

b) Changes in EMG power spectrum

The changes in the motor unit action potential indicate changes in transmission of the motoneuron action potential, release of transmitter and propagation of action potential along the sarcolemma. Changes in MUAP will affect the frequency composition of the surface EMG recording. The frequency composition of EMG is also affected by multiple other factors including firing rates of the various motor units, interference from the other action potentials, distance of the various muscle fibres from the recording electrode and placement of electrodes with respect to the neuromuscular junction. To analyse changes in the frequency composition of surface EMG, changes in the EMG power spectrum was investigated.

Power spectra were computed from EMG data obtained from the initial and final 30 seconds of a run. Median power frequencies of each record were then computed (see methods) (detailed data tabulated in Appendix G). Initial mean MPF values (initial MPF 131.50 ± 21.16 Hz), were found to be greater than the final mean MPF values (final MPF 124.83 ± 24.06 Hz), indicating a decrease in MPF over time. This change was statistically significant (paired t-test, $t_{60} = 6.289$, $p < 0.001$). The data are plotted on Fig. 24 for 60 motor units.

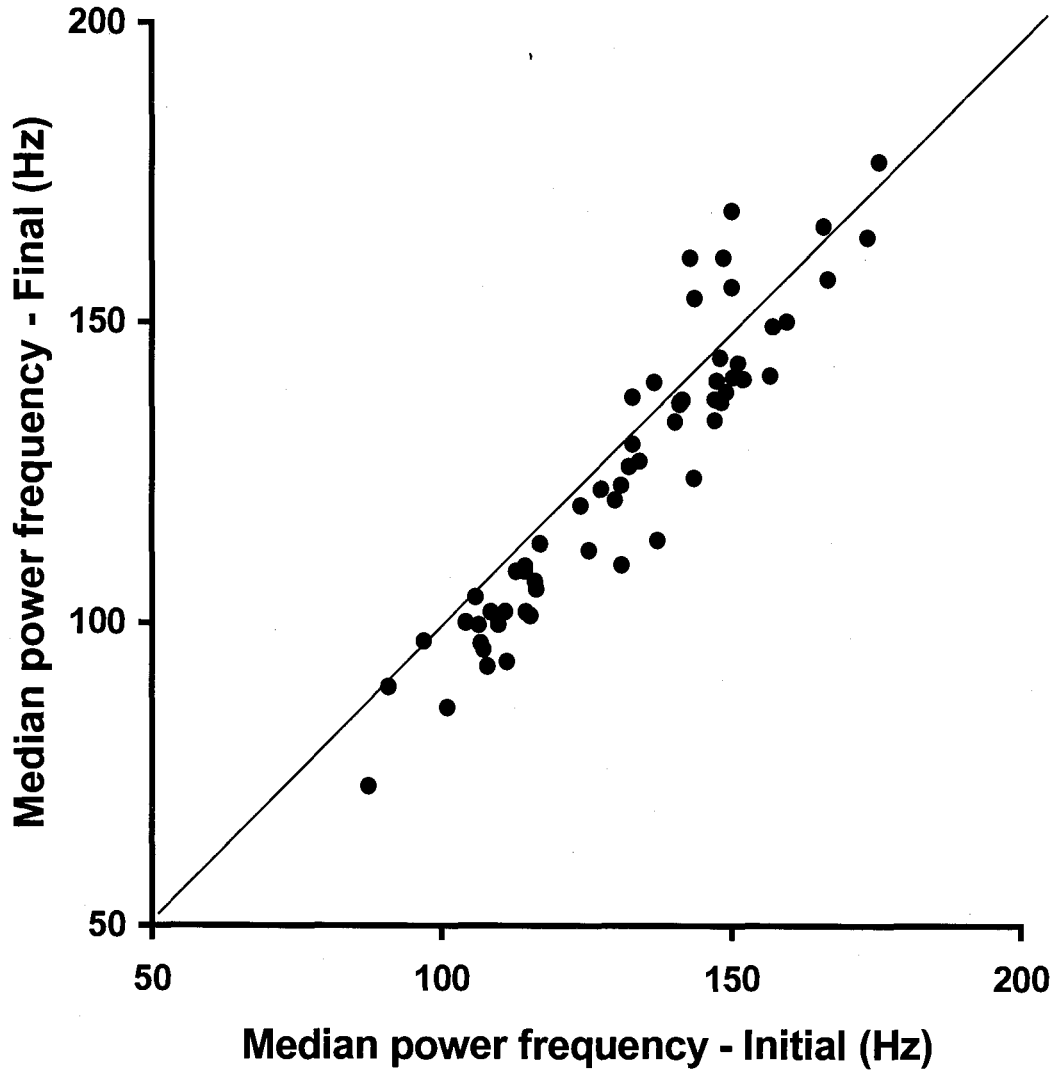

Figure 24: Changes in the median power frequency. The initial MPF values are plotted along the abscissa and corresponding final values are plotted along the ordinate.

Data were also analyzed to see if such changes were different for the fast and slow firing motor units. The change in MPF was 8.78 ± 5.34 % for the fast units and 1.94 ± 7.12 % for the slow units. The difference in percent change between the fast and slow motor units was statistically significant (independent t-test, $t_{37} = 1.687$, $p < 0.001$). The relationship between the fast and the slow motor units is presented in Fig. 25.

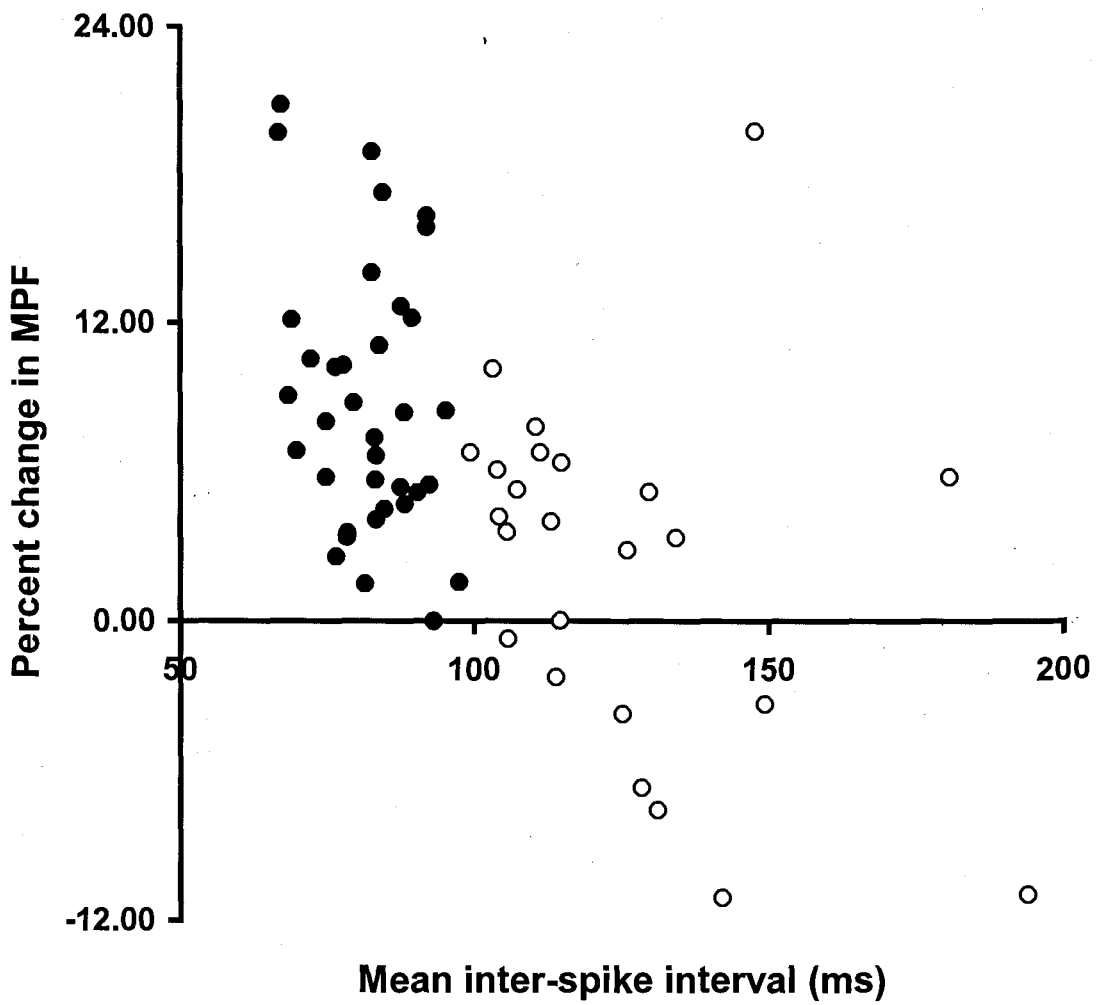

Figure 25: This figure represents the dependence of percent changes in MPF on ISI. The ISI of the motor units is presented along the abscissa and the percent change in MPF along the ordinate. Closed circles represent fast motor units, while open circles represent slow motor units.

Magnitude of fluctuations in force

Under normal conditions, certain amount force fluctuations can be observed in a muscle contraction. To investigate whether the magnitude of fluctuations in force increase with prolonged contraction, the standard deviation of force fluctuations of AC force was computed for the initial and final periods. On analysis of AC force fluctuations of 29 units, an increase in standard deviation was observed from 12.50 ± 14.41 grams to

23.44 ± 32.92 grams. This increase in standard deviation of AC force was statistically significant (paired t-test, t_{28} = -2.541, $\dot{p}$ = 0.017). The relationship between the force fluctuations from initial to final is presented on Fig. 26 (detailed data tabulated in Appendix H).

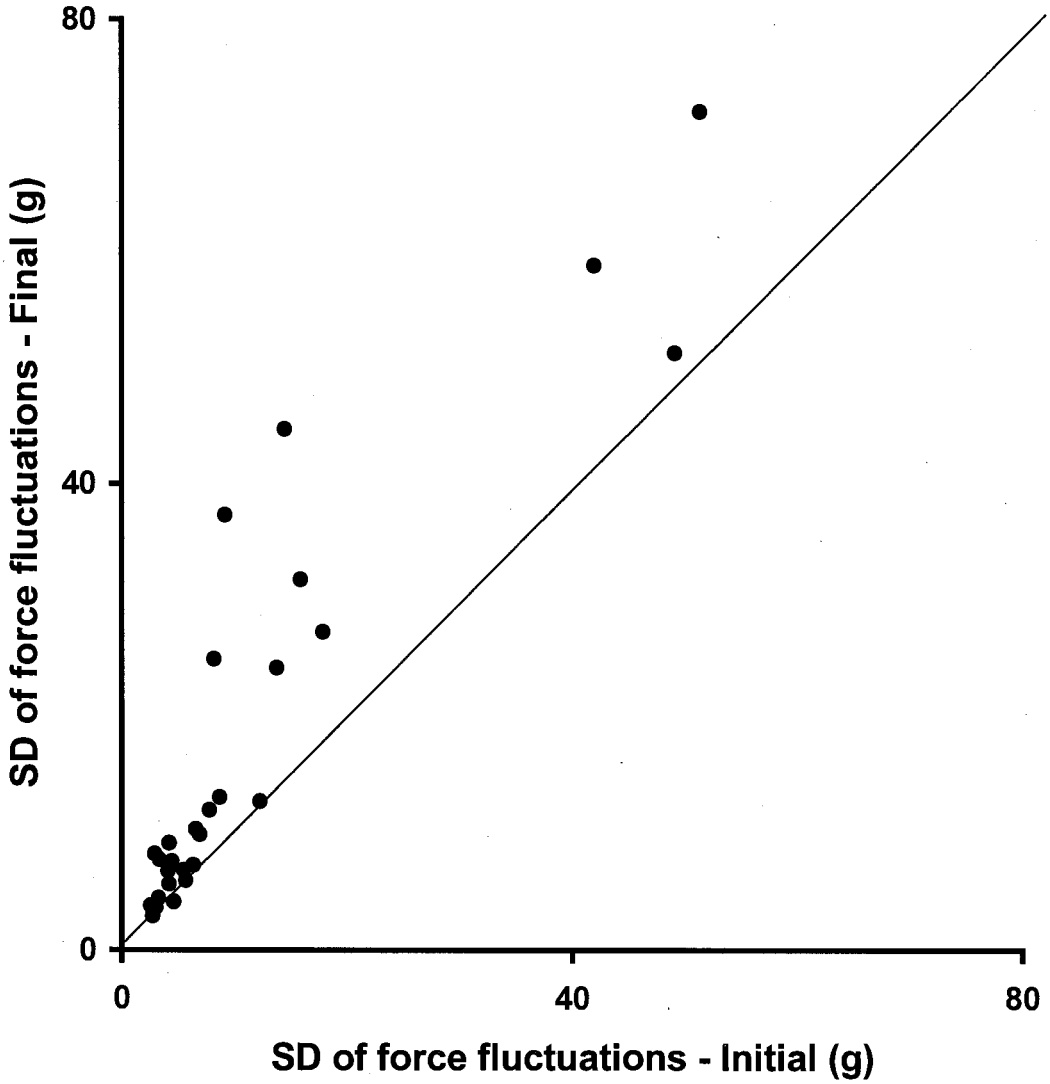

Figure 26: Changes in fluctuations of AC force. The initial standard deviation of AC force is plotted along the abscissa with the final standard deviation of AC force along the ordinate.

The standard deviation for the fast units had a mean increase of 104.88 ± 95.50 %, while the slow motor units had a mean increase of 34.39 ± 26.67 %. The difference between the fast and slow motor units with respect to change in the magnitude of AC force fluctuations was statistically significant (independent t-test, $t_{21} = 1.721$, $p = 0.004$). The relationship between the fast and the slow motor units is presented on Fig. 27.

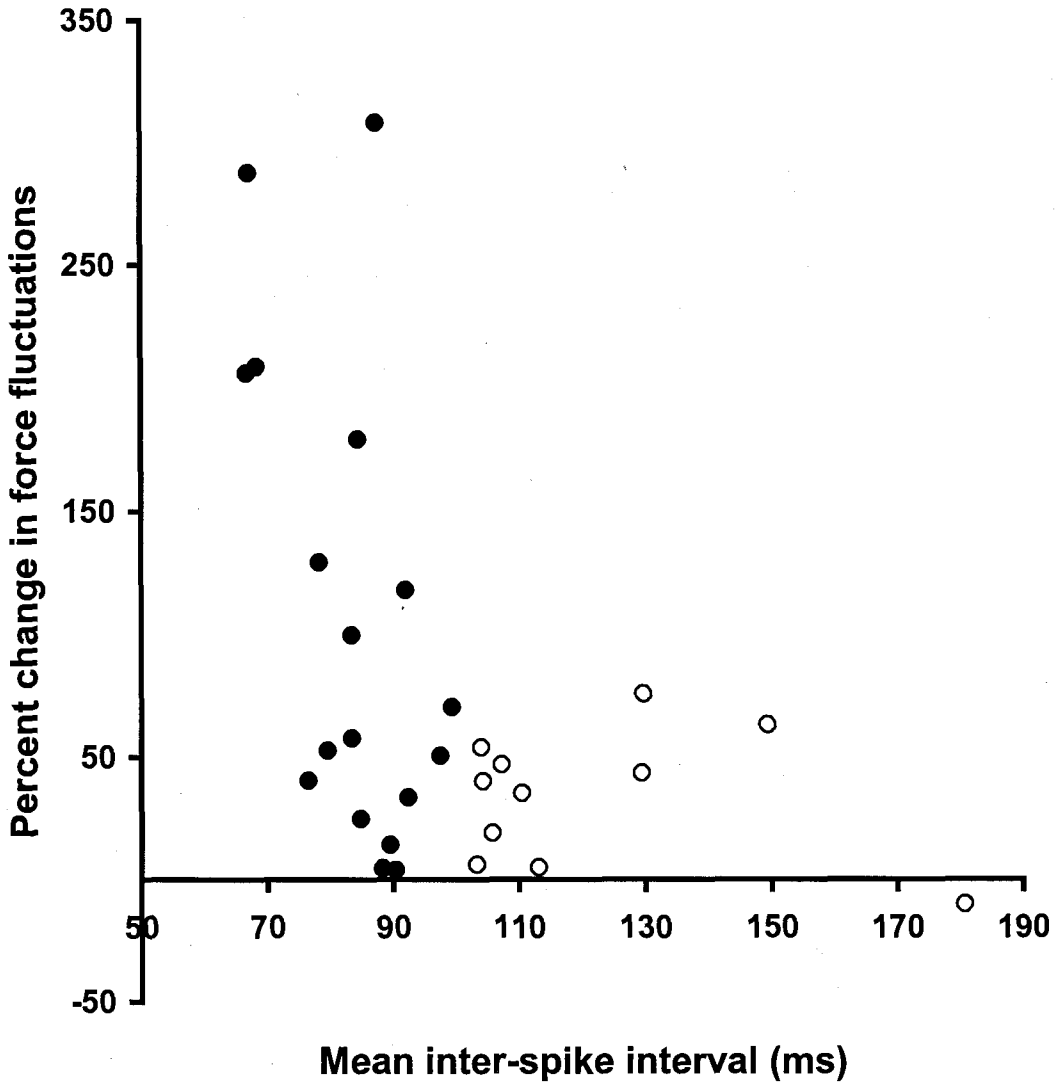

Figure 27: Figure represents the changes in the magnitude of AC force fluctuations. The ISI of the motor units is presented on the abscissa, with the percent change in force fluctuations on the ordinate. Closed circles represent fast motor units, while open circles represent slow motor units.

Relationship between targeted and non-targeted motor units

When subjects maintained firing rate of a well identified motor unit constant (targeted), some non-targeted motor units (concurrently firing motor units) were also recorded (Fig. 28). Since both motoneurons arise from the same motoneuron pool, observations were made to see the various differences between the two motor units when targeted motor unit was firing under controlled conditions. We wanted to examine if changes occurring in the targeted and non-targeted units were similar.

Slope and coefficient of variation were the two parameters based on which the comparisons were made (data presented in Appendices H and I). No significant differences were observed between the slopes of targeted and non-targeted motor units (paired t-test, $t_{16} = 1.75$, $p = 0.31$), indicating that both units in each pair maintained their respective firing rate, probably due to the kind of input received by both units. This relationship between the targeted and non-targeted units on comparing the slope data is presented on Fig. 29.

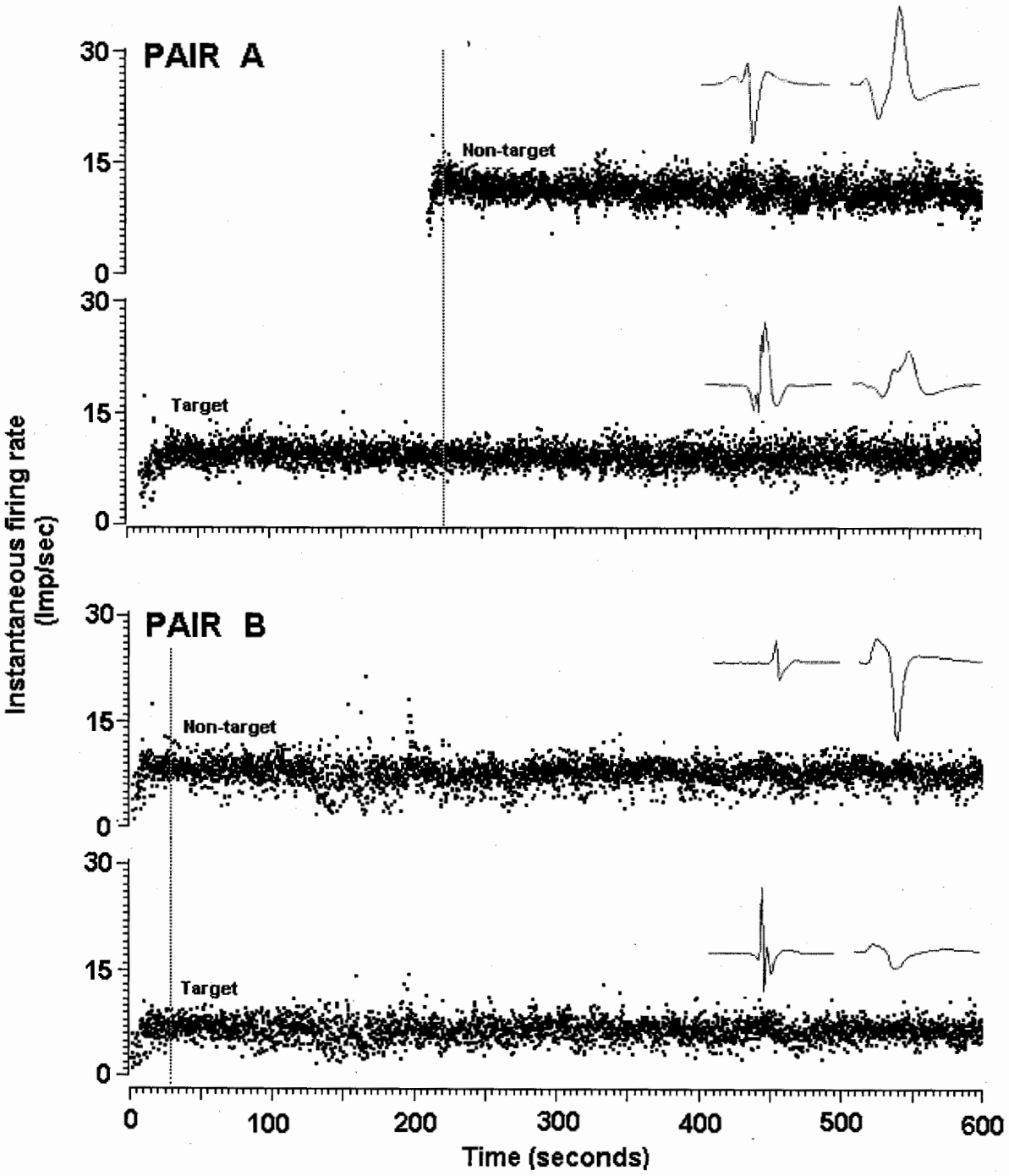

Figure 28: Figure denoted two pairs (Pair A and B) of motor units firing concurrently. The top figure (Pair A), denotes the firing of a non-targeted motor unit that was recruited during thew course of firing of the targeted motor unit. The motor unit triggered average from both the SMU and EMG recording are given indicating that both units are different from each other. The bottom figure (Pair B) denoted an example of a pair of motor units that were firing concurrently from the beginning. Again both the motor unit triggered averages from both the SMU and EMG recording are given to differentiate between the two units.

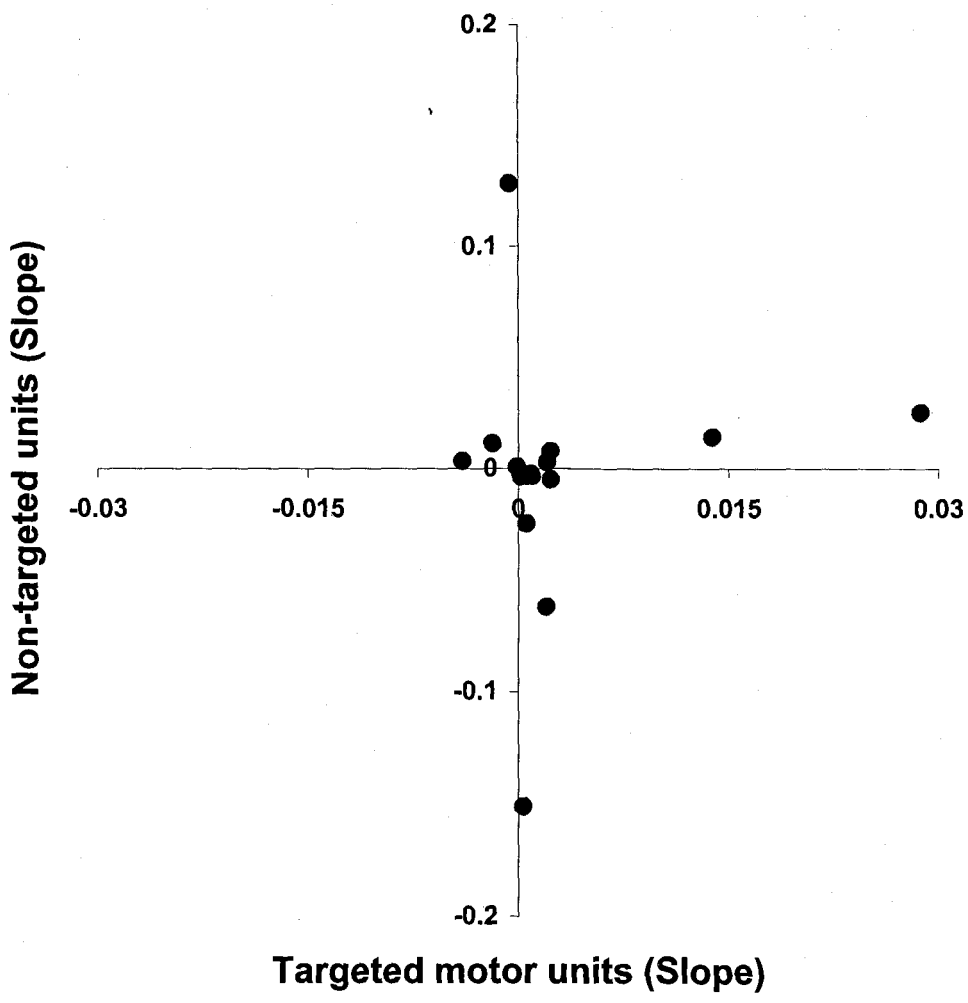

Figure 29: Relationship on slope of the firing distribution between the targeted and non-targeted motor units.

On comparing variability of ISI (coefficient of variation) between targeted (mean ± SD = 33.79 ± 69.36 %) and non-targeted (mean ± SD = 5.58 ± 38.29 %) motor units no significant differences were observed (independent t tests. $t_{16} = 1.71$, $p = 0.08$). Since both targeted and non-targeted motoneurons belonged to the same motoneuron pool, they can be assumed to receive the same amount of input signals. We have already shown that coefficient of variation for the targeted motoneurons increased by 31.75 % with prolonged firing. These results suggest that despite firing at different rates, the input to all

pairs of motoneurons were the same. The relationship between the coefficient of variation of targeted and non-targeted units is plotted on Fig. 30.

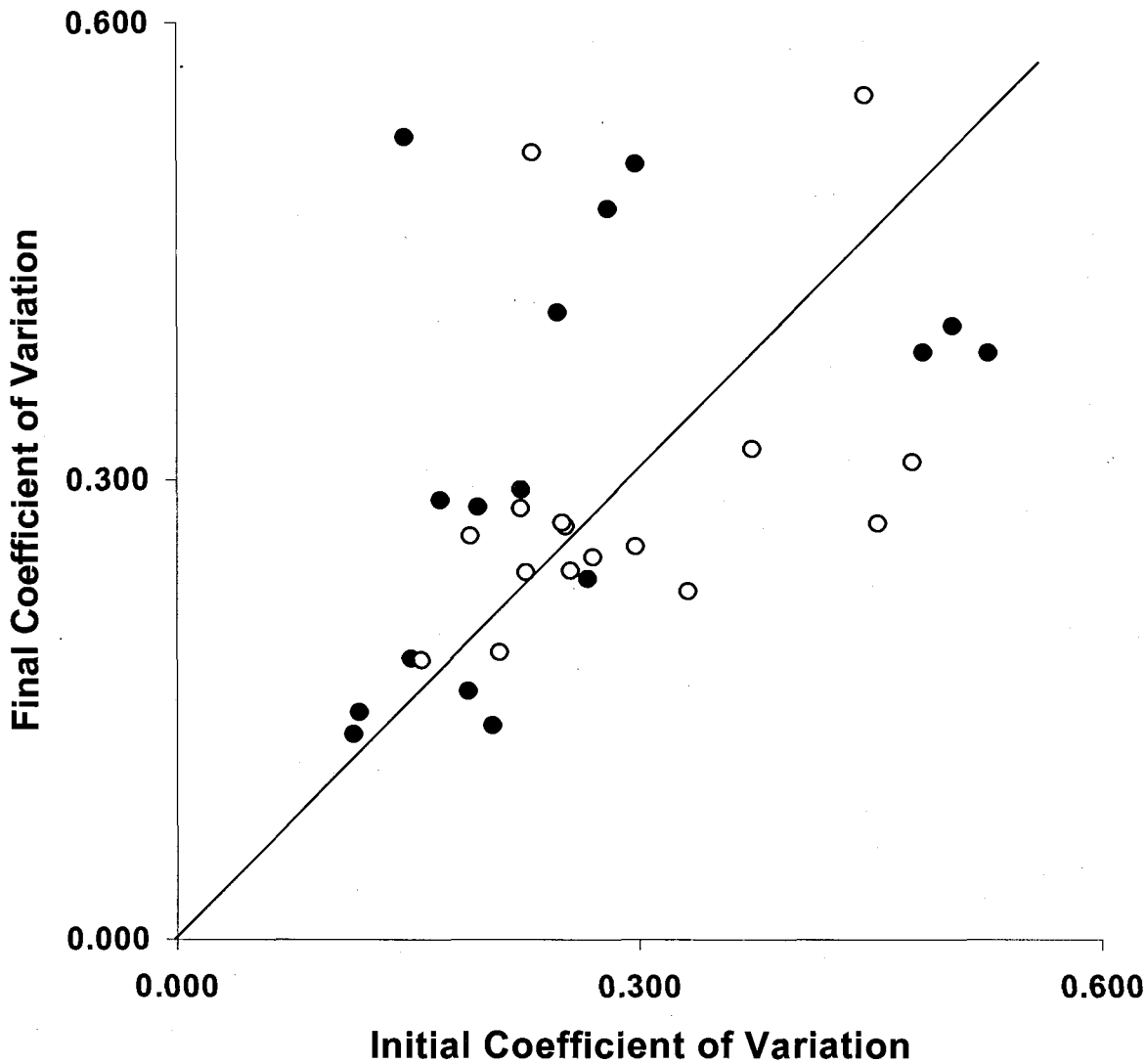

Figure 30: Relationship of coefficient of variation between targeted and non-targeted motor units. The closed circles represent targeted units, and the open circles represent accompanying non-targeted units.

As mentioned in methods, some non-targeted units fired from the beginning (category 1, N = 9 pairs) while others were recruited later on (category 2, N = 7 pairs) during the firing of the targeted motoneuron (detailed data tabulated in Appendix I and J).

For those pairs of motor units in category 1, no differences were observed in the slope between the targeted and non-targeted units (independent t-test, $t_8 = -0.37$, $p = 0.36$), indicating that the non-targeted motoneurons maintained their firing rate similar to that of the targeted motoneurons. This relationship between the slopes of the two categories is plotted on Fig. 31.

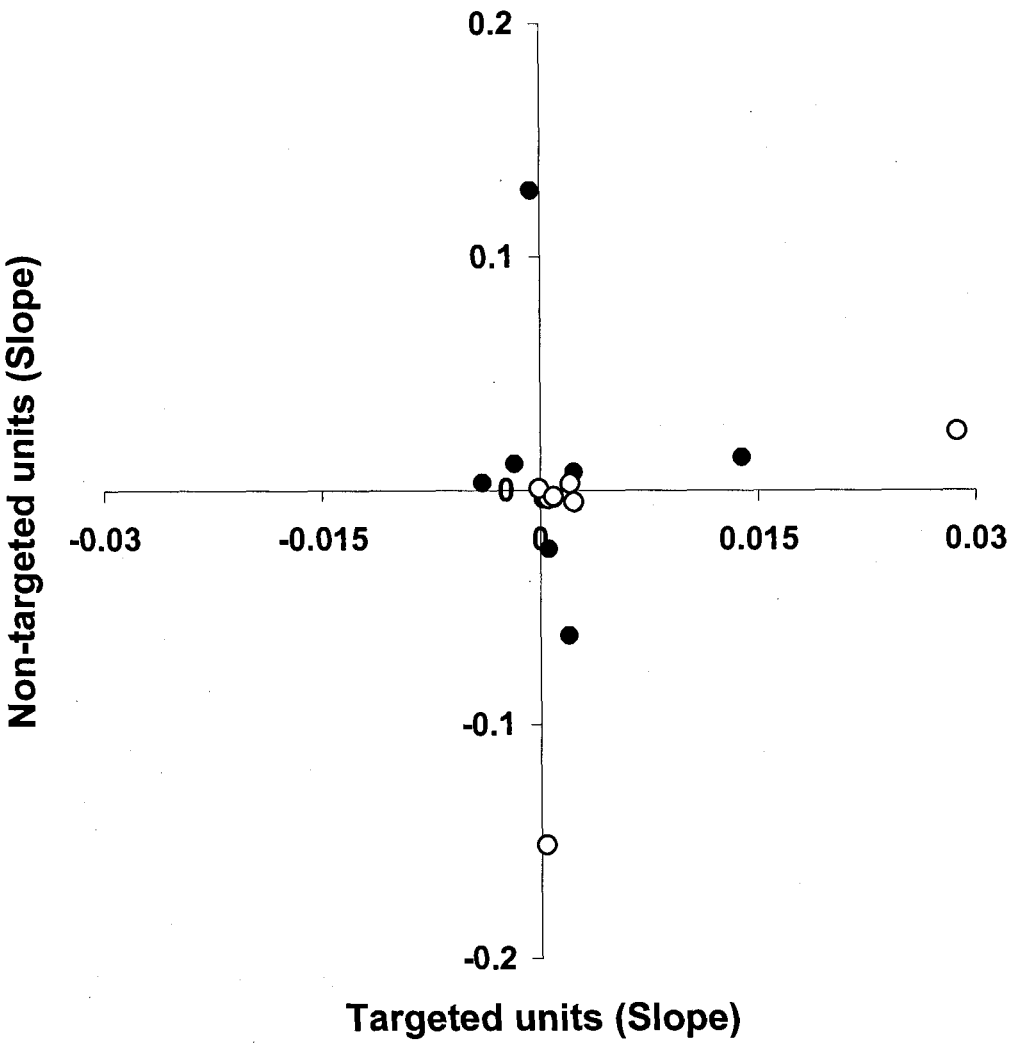

Figure 31: Comparison of the slope between the targeted and non-targeted motor units for both categories. The closed circles represent category 1 and the open circles represent category 2.

Similar to the pairs of units in category 1, units in category 2 also did not have any significant difference between the slopes of the targeted and non-targeted units (independent t-test, $t_6 = 1.06$, $p = 0.16$), probably indicating similar input signals. This relationship of coefficient of variation between the two categories is plotted on Fig. 32.

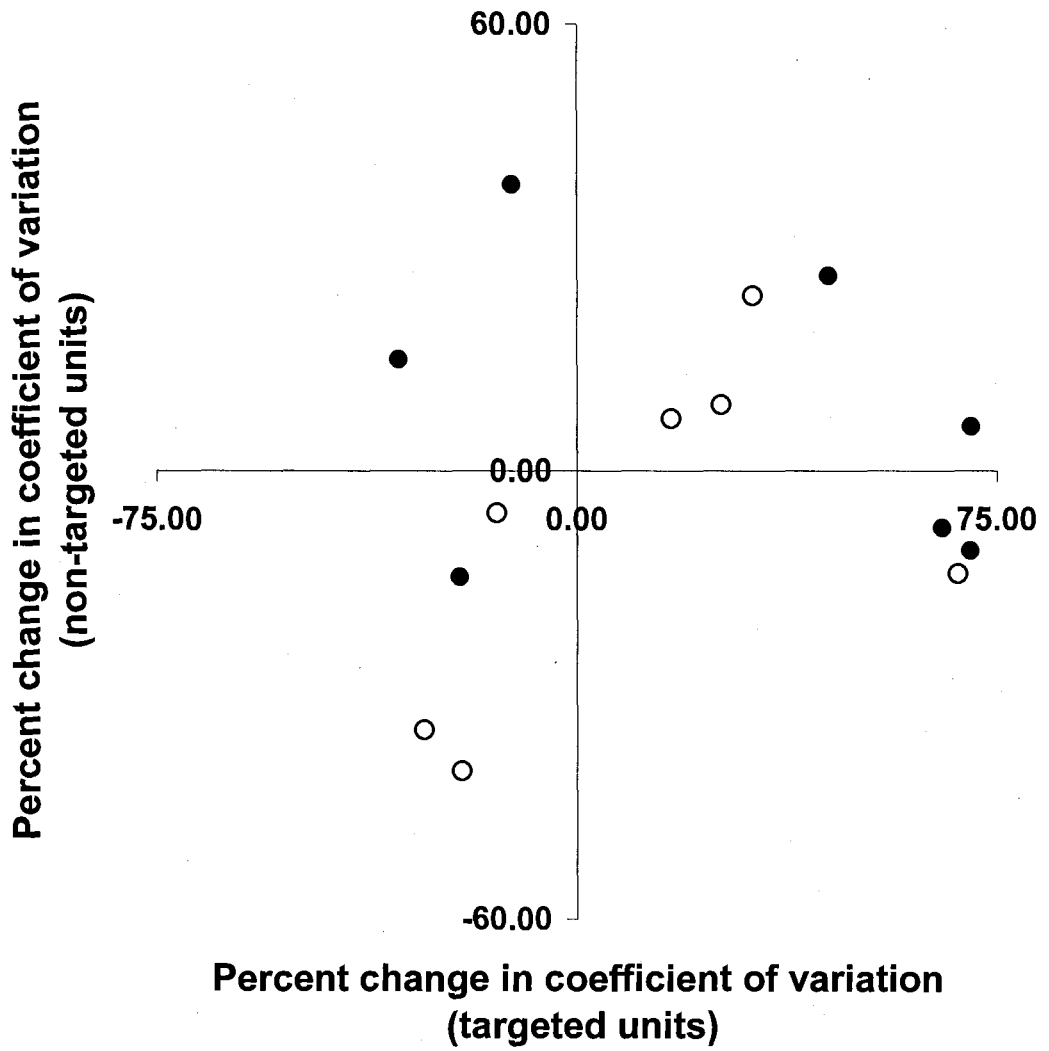

Figure 32: Comparison of the percent change in coefficient of variation between the targeted and non-targeted motor units for both categories. The closed circles represent category 1and the open circles represents category 2.

Comparison between pairs of motor units based on their firing rates

Among all analyzed motoneuron pairs, for 9 pairs the targeted units were faster than non-targeted units, while the reverse was true for the remaining 7 pairs. Analyses were done to observe changes between slope for both groups. The pairs with targeted units firing at higher rates than non-targeted units, the targeted units had a mean change in slope of 0.005 ± 0.010 while the non-targeted units had a mean change in slope of -0.005 ± .074. This difference in slope between the two groups was not significant (independent t-test, $t_8 = 0.433$, $p = 0.34$). This relationship is presented as 'A' on Fig. 33.

For the group with non-targeted units firing at higher rates than targeted units, targeted units had a mean change in slope of $7.09\ e^{-05} \pm 0.002$ while the non-targeted units had a mean change in slope of $-1.98\ e^{-03} \pm 0.012$. No significant difference in slope was observed between the two groups (independent t-test, $t_6 = 0.453$, $p = 0.33$). This relationship is presented as 'B' on Fig. 33.

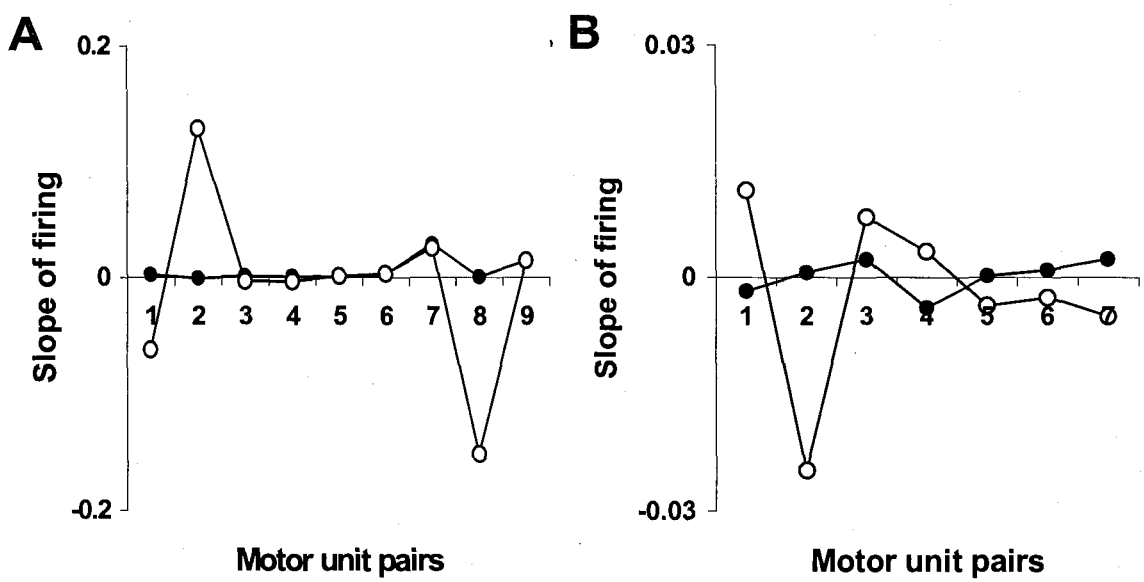

Figure 33: Figure A shows the slope relationship between the motor units, where the targeted units were faster than the non-targeted units. Figure B indicates the slope relationship between motor units, where the non-targeted units were faster than the targeted units. For both figures, closed circles represent targeted units, and open circles represent the non-targeted units.

Comparisons between targeted units and non-targeted units were done to look at differences in coefficient of variation. The pairs where the targeted units were faster than the non-targeted units, targeted units had a mean change in coefficient of variation of 40.74 ± 86.12 % while the non-targeted units had a mean change in coefficient of variation of 4.99 ± 50.30 %. No significant difference was observed between the two groups (independent t-test, $t_{13} = 1.08$, $p = 0.15$). This relationship is presented as 'A' on Fig. 34.

For the pairs where the non-targeted units were faster than targeted units, targeted units had a mean change in coefficient of variation of 24.85 ± 44.4 % while the non-targeted units had a mean change in coefficient of variation of 6.33 ± 17.03 %. No statistically significant differences were observed between the two groups (independent t-test, $t_8 = 1.03$, $p = 0.17$). This relationship is presented as 'B' on Fig. 34.

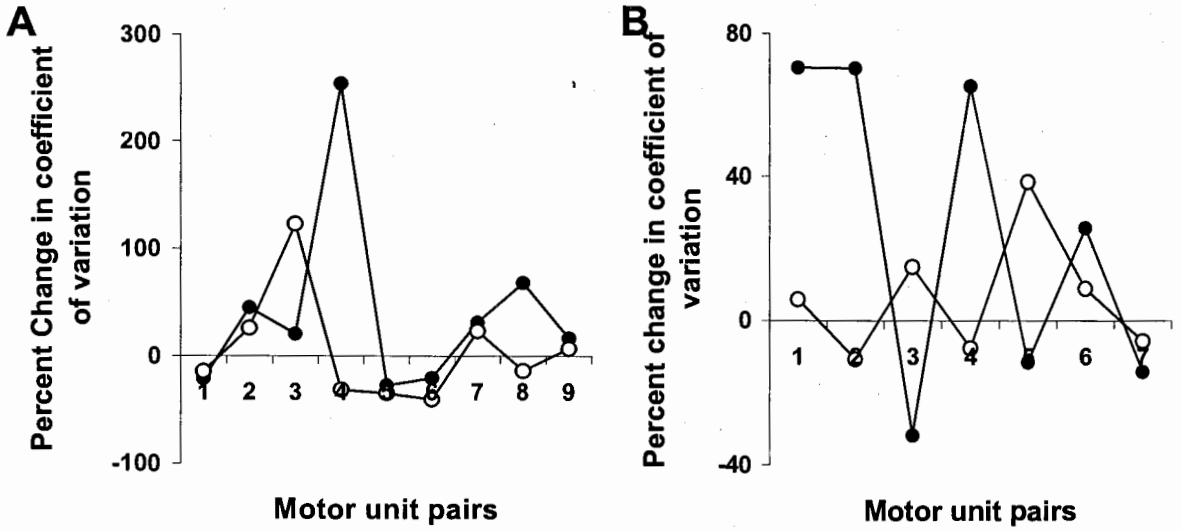

Figure 34: Figure A shows the relationship of coefficient of variation between the motor units, where the targeted units were faster than the non-targeted units. Figure B indicates the relationship of coefficient of variation between motor units, where the non-targeted units were faster than the targeted units. For both figures, closed circles represent targeted units, and open circles represent the non-targeted units.

DISCUSSION

The novel result in this thesis is that motoneurons fatigue on prolonged firing. This was reflected by an increase in EMG with prolonged firing. Related changes in the coefficient of variation of the inter-spike interval, median power frequency, motor unit potential and the behaviour of concomitantly firing motor units have previously been reported in the literature. Our results agree with some observations and disagree with the others. The details of these observations are discussed below.

Threshold of motoneuron discharge and surface EMG

When a motoneuron discharges for a prolonged period, the results demonstrate that EMG increases while firing rate of the motoneuron is maintained. In general EMG is an indicator of total electrical activity of muscle that includes number, size and the respective firing rates of individual motor units (Sanders et al., 1996). Since the rate was constant in our experiment protocol and the magnitude of EMG increased significantly, it would imply that the number of active motor units / motoneurons increased. This could happen only if the targeted motoneuron required additional excitatory input to maintain its firing rate or the discharge threshold of the motoneuron increased with prolonged firing. This increase in discharge threshold will be called "motoneuron fatigue". Since the non-discharging motoneurons are not fatigued, the additional excitatory input is sufficient to recruit them, and hence, there is an increase in EMG.

Fatigue of motoneurons has not been directly demonstrated in human experiments. In cat experiments (Kernell, 1965a, b, c; Baldissera, 1982; Sawczuk, 1995)

and in human experiments (Smith et al., 1995) it has been shown that motoneuron discharge rate decreases with time while magnitude of input current is maintained for approximately 60 s. This would imply that during adaptation the motoneuron would require additional input in order for it to maintain a constant firing rate. In cat (Baldissera, 1987) and in human experiments (Smith et al., 1995) it has been shown that the magnitude of adaptation is directly proportional to the rate of change of input current. In our experiments very little adaptation was observed, as the rate of change in force was comparatively low (see firing rate profile in Figs.14 and 28). So the mechanisms do not explain the increase in EMG. Another term that is used to indicate changes in motoneuron discharge threshold is accommodation. When the depolarization increased slowly, voltage gated Na^+ channels open and inactivate. As slow depolarization proceeds, more and more Na^+ channels inactivate. A prolonged depolarization would inactivate more Na^+ channels, and to keep the motoneuron firing, additional excitatory input is required. Mechanisms underlying late adaptation suggested by Sawczuk et al. (1995) could be responsible for the "motoneuron fatigue" observed in our experiments. Both reflect slow increases in discharge threshold. In addition to progressive increase in Na^+ inactivation, those authors also suggest a slow increase in outward current thus leading to hyperpolarization of the soma and initial segment. This would also increase threshold. In addition to these channels, changes in energy stores have been shown to occur in neurons (Bernstein and Bambur, 2003).

Some of the previous reports on the decrease in firing rate during sub-maximal contractions have suggested a decrease in the net excitatory input to the motoneuron pool. The decrease occurs in descending inputs (Taylor et al., 2000) and reflex feedback

(Bigland-Ritchie et al., 1986a; Woods et al., 1987; Garland, 1991). All these experiments were done with constant force paradigms. Only one set of experiments (Nordstrom and Miles, 1991a,b) used constant firing rate paradigm, but the authors never addressed the question of 'motoneuron fatigue'. Our results clearly indicate that in addition to a decrease in net excitatory input, fatigue of the motoneuron itself is an additional factor that decreases firing rate.

We have also shown that the increase in EMG was higher for the fast firing units despite the fact that those units, on the average, fired for shorter duration. A higher firing rate results from a higher magnitude of membrane depolarization. The higher fatigue may result from the higher levels of depolarization and hence the inactivation of Na^+ channels. In addition it may result from the generation of action potential itself, which would contribute to inactivation of Na^+ channels, accumulation of Ca^{2+} and increased K^+ efflux.

Statistical properties of motoneurons

Under normal conditions the firing rate of a tonically firing motoneuron shows variability around a mean. This variability arises mostly from synaptic noise (Calvin and Stevens, 1967, 1968; Jones and Bawa, 1997). As mentioned in the Introduction, variability of inter-spike interval increases with increasing ISI (Person and Kudina, 1972; Jones an Bawa, 1997). During our experiments when we kept the firing rate constant, variability in ISI value increased with prolonged firing as shown by an increase in coefficient of variation at every firing rate tested. Increase in variability has been shown during fatigue experiments when force was maintained constant (Gantchev et al., 1986;

Enoka et al., 1989). Increase in variability has also been shown to increase using constant firing rate protocol on the human masseter muscle (Nordstrom and Miles, 1991a,b). Therefore whether it is "constant force" or "constant firing rate" paradigm, both show an increase in coefficient of variation of ISI. (Person and Kudina, 1972; Gantchev et al., 1986; Enoka et al., 1989; Nordstrom and Miles, 1991a,b; Sturm et al., 1997). Calvin and Stevens (1967, 1968) have shown that the main contributor to noise of the motoneuron membrane is the synaptic input. An increase in membrane noise would mean an increase in the amplitude of membrane noise. This can result from two sources. The first source is that the synaptic inputs are becoming more synchronized to result in larger amplitude of noise. This view is supported by the suggestion that synchronization of motor units increases with fatigue (Arihara and Sakamoto, 1999; Kleine et al., 2001). The second possibility is that the motoneuron membrane becomes more sensitive to inputs during prolonged depolarization. Though it is a speculation, the increased sensitivity could mean larger number of ligand gated channels responding to the transmitter or larger currents flowing through each channel, or new type of channel opening with prolonged firing. For example, initial depolarization may open only AMPA type glutamate channels, while prolonged firing may lead to opening of NMDA channels, which have a much higher conductance. This increased sensitivity of the cell body and dendrites is not a contradiction of the increased firing threshold that occurs at the initial segment.

The percentage change in the coefficient of variation in the fast firing and the slow firing units was the same. As mentioned in the Results, fast firing units fired for a shorter period than the slow firing units. The two groups may have fired, on the average,

the same number of action potentials, thus exhibiting the same amount of changes responsible for noise.

Changes in muscle unit action potential

Under normal conditions, an action potential triggered at the initial segment of the motoneuron results in contraction of corresponding muscle unit. All muscle fibres of a motor unit fire almost synchronously. The compound action potential recorded during simultaneous discharge of all muscle fibres is called motor unit action potential (MUAP). Surface EMG electrodes record the total electrical activity of the muscle. Muscle fibres that are close to the surface contribute larger currents to EMG, and the contribution decreases as the distance between muscle fibres and the surface where recording electrodes are placed increases (Stalberg et al., 1996). Each motor unit contributes to surface EMG. Since muscle fibres of a motor unit are distributed throughout the muscle, there is a different amount of contribution by each muscle fibre to surface EMG.

In humans MUAP cannot be recorded when multiple units are active. One can get an estimate of this parameter from surface EMG. A single motor unit spike, recorded with intramuscular microelectrodes, represents the activity of only a few muscle fibres of a motor unit. One can use this spike to trigger averaging of surface EMG to obtain an estimate of MUAP. It should be mentioned that the estimate of MUAP depends on the electrode size and positioning. However, changes occurring in the profile of MUAP during one experimental session are not prone to such errors.

When a motoneuron fires for prolonged periods, various changes occur along the path of the action potential including the terminal arbourizations of the motoneuron,

neuromuscular junction and sarcolemma. Such changes will be reflected as widening of the action potential. Analysis from our study revealed a significant increase in duration of the muscle unit action potential. This was consistent with results reported in the literature (Burke et al., 1973; Sandercock et al., 1985; Conwit et al., 1999). Both Burke et al. (1973) and Sandercock et al. (1985) studied changes on medial gastrocnemius of anaesthetized cats using different frequencies of electrical stimulation's (Burke et al., used 40 Hz, Sandercock et al., used 10, 40 and 80 Hz). Based on their work, they suggested these changes are due to failure at axonal branch points, increased jitter in release and uptake of Ach, neuromuscular junction and / or a decrease in the impulse propagation along sarcolemma and/or T-tubules.

In humans, Conwit et al. (1999) reported an increase in the area of surface detected MUAP and increase in the amplitude of MUAP (Conwit et al., 2000). These authors suggested that the changes were due to an increase in recruitment of additional motor units due to fatigue. These are the only two studies known to the author, which are shown to demonstrate an increase in MUAP amplitude and MUAP area. In comparison, our results indicated significant increase in MUAP duration with no significant changes in MUAP amplitude.

Changes in duration of MUAP can occur due to a number of factors. Firstly, this may be due to hyperpolarization of the motoneuron axon when motoneuron fires for prolonged periods (Vagg et al., 1998; Kubawara et al., 2000). Hyperpolarization of an axon will decrease the action potential conduction velocity that will result in greater dispersion of action potentials among the hundreds of terminal boutons. Hyperpolarization will also affect the release of ACh. On the post-synaptic side, similar

mechanisms could lead to slowing of conduction velocity along the sarcolemma resulting in increased MUAP duration. Reports from the literature indicate a decreased excitability of muscle membrane along with decreased impulse propagation velocity (Jones et al., 1979; Milner-Brown and Brown, 1986). The dispersion of action potentials pre-synaptically and slowing of conduction velocity along the sarcolemma would lead to an increase in the duration of MUAP. We did not observe any significant changes in the MUAP amplitude. This would imply that all muscle fibres of the motor unit were activated until the end.

Changes in EMG power spectrum

Under normal conditions the EMG recorded using bipolar surface electrodes contains electrical activity recorded of all active muscle fibres. EMG is the interference pattern generated by the asynchronous activity of multiple units. EMG contains information on the shape and size of each MUAP, the number of active motor units and their firing rates (Bilodeau et al., 1994). Since EMG is an interference pattern, the very high frequencies are filtered out. Furthermore, the various tissues, including the skin through which current has to travel to reach the recording electrodes, filter high frequencies. If one compares the power spectrum of EMG and power spectrum of an action potential of a muscle fibre, one can see that the latter contains frequency well above 10 KHz while EMG does not. The peak power of EMG lies in the range of 100 – 500 Hz. Still, during any one experiment, changes in frequency composition of EMG will reflect changes in MUAP, firing rate and recruitment of new units (Bilodeau et al., 1994).

Results from our study indicated a statistically significant decrease in median power frequency, when compared between the initial and final periods of a run. These results were found to be consistent with observations reported in some of the previous literature (Petrofsky et al., 1982; Eberstein and Beattie, 1985; Fuglevand et al., 1993; Bilodeau et al., 1994; Perry et al., 2001), while it contradicted the others (Petrofsky, 1979; Jensen et al., 2000).

The power spectrum of EMG showed a shift towards lower frequency. An increase in the duration of MUAP will shift the spectrum towards lower frequency, so will the firing rates of newly recruited units. On the other hand, the MUAPs of newly recruited units will add to higher frequencies. An increase in muscle temperature will shift the spectrum towards high frequencies (Petrofsky, 1979). It appears the latter two factors added less to our spectrum than the former two factors.

Though our results agree with the results reported in the majority of the literature (Petrofsky et al., 1982; Eberstein and Beattie, 1985; Fuglevand et al., 1993; Bilodeau et al., 1994; Perry et al., 2001), the contrasting results may be due to differences in experiment protocols or the muscle. For example, Petrofsky et al. (1979) demonstrated an increase in MPF during VO_2 max tests. An increase in temperature increases the conduction velocity of an action potential, thus adding to the high frequencies. Temperature has not been controlled in majority of the experiments including ours. It was difficult to insert two needles in a small muscle without causing discomfort to the subject. We did record surface temperature in a few experiments. The net rise in temperature depended on the subject. Since the surface recorded temperature is not a true indicator of intramuscular temperature, we did not pursue recording temperature.

Changes in fluctuations of AC force

Under normal physiological conditions, force is produced by muscle contraction. This is achieved by asynchronous firing of multiple motor units. Though the asynchronous firing facilitates smooth force production, some amount of fluctuation in force production is still present. By filtering the DC component of the total force, one can observe the characteristics of the fluctuations in the resultant AC force. We measured the standard deviation of AC force and demonstrated a significant increase in the standard deviation of AC force during prolonged contraction. Our analysis is different from the other studies dealing with force fluctuations. Previous studies dealt with changes in the magnitude of physiological tremor (8-12 Hz) with fatigue (Arihara and Sakamoto, 1999; Löscher et al., 1996).

An increase in magnitude of tremor is attributed to synchronization of motor units and oscillations of the stretch reflex loop (Arihara and Sakamoto, 1999). The same mechanisms probably explain our observations. We have not restricted our analysis to only the physiological tremor but also to the whole range of 1-50 Hz. Increases in the variability of ISI of each motor unit will increase the force fluctuations of that unit irrespective of the presence of stretch reflex feedback. If force output of each motor unit exhibits more fluctuations, the total force will exhibit increased amplitude of fluctuations. This will lead to a decreased control of force or the accompanying movement. Increased synchronization during fatigue reported by (Arihara and Sakamoto, 1999) will also contribute to increased fluctuations.

Firing characteristics of targeted and non-targeted units

Under normal conditions multiple motoneurons are excited to perform a given activity. The number of active motor units depends on strength of a given activity. Mostly these units fire asynchronously (see Introduction) with respect to each other. Our observations concentrated on the firing behavior of a pair of motor units, when the firing rate of one (targeted unit) was maintained at a constant rate.

Results from our study indicated that when the firing rate of the targeted unit was kept constant, the firing rate of the non-targeted unit also stayed the same. There was no difference in the change in the coefficient of variation between the two units. Comparisons were also made between the units that fired from beginning as opposed to units that were recruited later to see if time of recruitment would make any difference. Analysis revealed no significant difference. Further comparisons were made to compare units that fired at different firing rates. In some cases the targeted units fired at rates higher than the non-targeted units and vice versa in others. These comparisons failed to reveal any significant differences. Based on our findings all analysis on the comparisons between the targeted and non-targeted units revealed no significant difference between the behavior of targeted and non-targeted units. These results were consistent with reports from DeLuca and Erim (1994, 2002). DeLuca and Erim (1994, 2002) showed a parallel increase and decrease of firing rates of concomitantly firing motor units. These results are interpreted to show the presence of a common drive to all motoneurons resulting in parallel changes in all motoneurons.

Nordstrom and Miles (1990) on the other hand showed increase in firing rate of some non-targeted units and decreases in firing rate of other non-targeted motor units of masseter muscle. One possibility in the difference is that the masseter is a complex muscle; therefore the motoneuron pool may be controlled differently from the motoneuron pool of a relatively simple limb muscle. Other possibilities are the initial firing rates of the non-targeted units. If the initial firing rate was very high, they may adapt. If the initial firing rates were very low, they will increase to attain a steady rate. In our analysis, we by-passed these initial changes and analyzed the firing rate behavior only after steady firing rate had been achieved.

From the reports from other literature, it is evident that there must have been a common input that excited both the targeted and non-targeted units.

Conclusion

The main objective of this thesis was to determine if motoneurons fatigue on prolonged firing. For this purpose an experimental protocol was designed to examine the presence of 'fatigue' in human motoneurons. We used constant firing rate protocol to reach our goal. The results clearly showed a significant increase in EMG when a targeted motoneuron was maintained at a constant firing rate. This implies that the motoneuron required increased excitatory input in order to keep firing at the same rate for a long time. Along with this a significant increase in ISI variability was observed indicating a significant increase in synaptic noise/inputs.

In this study, motoneurons were maintained at a constant firing rate and possibly due to some intrinsic changes motoneurons required additional excitatory drive to

maintain firing rate, indicating motoneuron fatigue. These changes were observed in various parameters that were monitored and analysed. Firstly, the motoneuron required additional input to maintain the firing rate. Secondly, though a constant firing rate was maintained, a significant increase in the coefficient of variation was observed indicating alterations to the input to the motoneuron that affects the motoneuron outputs. Changes in other parameters including the MUAP, MPF and increase in force fluctuations also sum up to confirm the fatigue in the motoneuron.

In future, these experiments performed in this study can be altered to accommodate more changes. Long duration muscle contractions, will help in more fatigue data. Higher firing rates would enable us to observe even greater changes both in ISI variability and alterations in force fluctuations. Monitoring intra-muscular temperature will increase our understanding of changes in EMG power spectrum. Also if compound muscle action potentials were monitored both before the start of a run and at the end, it will reveal the extent of changes at the junction and the sarcolemma.

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APPENDICES

Appendix – A

Ethics Compliance Documents

SIMON FRASER UNIVERSITY

OFFICE OF VICE-PRESIDENT, RESEARCH

BURNABY, BRITISH COLUMBIA
CANADA V5A 1S6
Telephone: (604) 291-4370
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November 22, 1999

Dr. Parveen Bawa
School of Kinesiology
Simon Fraser University

Dear Dr. Bawa:

Re: Input-Output Properties of Human Spinal Cord
NSERC

I am pleased to inform you that the above referenced Request for Ethical Approval of Research has been approved on behalf of the University Research Ethics Review Committee with the points of clarification you offered in your e-mail correspondence to Dr. Fattah on November 3, 1999. This approval is in effect for a period of three years from the start of the research project or for the term of your faculty appointment at SFU. Any changes in the procedures affecting interaction with human subjects should be reported to the University Research Ethics Review Committee. Significant changes will require the submission of a revised Request for Ethical Approval of Research.

Best wishes for success in this research.

Sincerely,

Dr. James R.P. Ogloff, Chair
University Research Ethics Review Committee

c: R. Marteniuk, Dean
/bir

Appendix – B

Data for general firing characteristics of motoneurons

File #	ISI - Mean (ms)	Onset duration d (seconds)	Total. Duration x (seconds)
VJ25FE26	66.7	10	245
JH27FE7	67.1	31	260
VJ23FE7	68.3	6	390
VJ18DC13	68.9	30	280
VJ13NV17	69.8	20	195
CV06OT26	72.3	12	260
JH24DC17	74.8	12	560
VJ10OT25	74.8	3	73
JH26FE7	76.5	5	255
JS06DC17	76.5	22	540
VJ09OT25	77.8	5	220
JH28FE26	78.3	13	530
VJ11OT25	78.5	13	83
VJ21FE7	79.6	12	121
JS05DC17	81.4	25	505
CV08OT26	82.6	20	140
VJ20DC21	82.7	54	630
VJ14NV17	83.1	13	90
JH19DC13	83.2	28	385
JS10FB27	83.4	22	300
VJ22FE7	83.4	13	150
JH22DC13	83.9	59	450
CV11NV16	84.5	40	675
JS04NV20	84.8	6	605
JH11OT29	87.5	15	545
VJ19DC21	87.7	8	605
VJ15NV17	88.1	15	120
JS07FE7	88.3	16	215
CV09NV16	89.5	15	535
CV01AU03	90.4	4	675
CV14NV19	92	12	545
VJ04JL13	92	29	635
JS09FB27	92.4	7	160
CV15DC21	93.1	35	395

File #	ISI - Mean (ms)	Onset duration d (seconds)	Total. Duration x (seconds)
VJ05JL17	95.3	27	645
JS11FB27	97.5	10	545
JH07SP11	99.4	41	589
VJ16NV17	103.3	17	675
CV03SP11	104	28	590
JS08FE7	104.3	18	445
VJ06JL17	105.6	21	595
JH03AU03	105.8	10	615
JH14NV16	107.3	12	515
JH17NV20	110.5	9	615
VJ02JL10	111.3	8	560
JO08NV17	113.1	60	380
VJ08JL20	114	36	675
JH02AU03	114.6	50	955
VJ03JL13	114.8	5	184
JO02AU03	125.2	75	160
JH12NV16	126.0	55	200
JO04AU03	128.5	26	465
JH09SP11	129.7	10	595
JH21DC13	131.1	29	395
JH01JL20	134.2	20	655
JH20DC13	142.1	4	405
VJ01JL10	147.7	61	1140
JS02NV20	149.3	5	575
JO07NV17	180.7	57	500
JO01JL20	194.1	40	850

Legend: Data of firing characteristics for all 60 recorded motor units. Data tabulated with the file number of each motor unit (column 1), the initial mean ISI (column 2), onset of firing duration (column 3) and total duration of firing (column 4). Data is tabulated for fast and slow units separately.

Appendix – C

EMG magnitude data for 58 motor units

File # Fast units	Initial mean ISI (ms)	Mean initial EMG (mV)	Percent MVC of initial EMG	Mean final EMG (mV)	Percent MVC of final EMG	Percent change in EMG
JH27FE7	67.1	0.114	9.66	0.187	15.87	64.42
VJ23FE7	68.3	0.033	4.42	0.083	11.11	151.20
VJ18DC13	68.9	0.049	6.48	0.074	9.85	51.94
VJ13NV17	69.8	0.079	10.53	0.105	14.04	33.40
CV06OT26	72.3	0.083	10.47	0.101	12.81	22.34
JH24DC17	74.8	0.036	3.03	0.049	4.12	35.77
VJ10OT25	74.8	0.023	3.01	0.053	7.07	135.09
JH26FE7	76.5	0.059	5.03	0.078	6.57	30.68
JS06DC17	76.5	0.067	6.77	0.064	6.47	-4.48
VJ09OT25	77.8	0.004	0.58	0.007	0.90	54.77
JH28FE26	78.3	0.057	4.83	0.072	6.12	26.66
VJ11OT25	78.5	0.076	10.15	0.094	12.51	23.31
VJ21FE7	79.6	0.023	3.01	0.043	5.78	92.00
JS05DC17	81.4	0.071	7.16	0.068	6.87	-4.08
CV08OT26	82.6	0.068	8.57	0.076	9.68	12.96
VJ20DC21	82.7	0.131	17.47	0.157	20.93	19.83
VJ14NV17	83.1	0.035	4.72	0.043	5.78	22.39
JH19DC13	83.2	0.054	4.57	0.060	5.06	10.75
JS10FB27	83.4	0.114	11.55	0.130	13.21	14.41
VJ22FE7	83.4	0.019	2.55	0.056	7.53	194.85
JH22DC13	83.9	0.056	4.71	0.067	3.11	19.97
CV11NV16	84.5	0.055	6.97	0.074	9.37	34.36
JS04NV20	84.8	0.054	5.47	0.056	5.67	3.66
JH11OT29	87.5	0.070	5.94	0.092	7.80	31.24
VJ19DC21	87.7	0.030	4.00	0.099	13.26	231.47
VJ15NV17	88.1	0.035	4.62	0.035	4.71	2.09
JS07FE7	88.3	0.054	5.51	0.044	4.48	-18.71
CV09NV16	89.5	0.077	9.71	0.087	11.01	13.32
CV14NV19	92.0	0.118	14.90	0.125	15.78	5.89
VJ04JL13	92.0	0.038	5.09	0.051	6.73	32.20
JS09FB27	92.4	0.104	10.59	0.122	12.35	16.63
CV15DC21	93.1	0.085	10.72	0.094	11.91	11.09
VJ05JL17	95.3	0.017	2.26	0.019	2.59	14.44
JS11FB27	97.5	0.169	17.20	0.219	22.27	29.47
JH07SP11	99.4	0.009	0.74	0.013	1.10	49.45
Average	**82.83**	**0.062**	**6.94**	**0.080**	**8.98**	**41.85**

File # Slow units	Initial mean ISI (ms)	Mean initial EMG (mV)	Percent MVC of initial EMG	Mean final EMG (mV)	Percent MVC of final EMG	Percent change in EMG
VJ16NV17	103.3	0.024	3.19	0.028	3.71	16.28
CV03SP11	104.0	0.017	2.18	0.032	4.00	83.53
JS08FE7	104.3	0.077	7.79	0.087	8.84	13.44
VJ06JL17	105.6	0.015	2.06	0.027	3.61	75.89
JH03AU03	105.8	0.006	0.55	0.012	1.00	82.27
JH14NV16	107.3	0.031	2.66	0.035	2.94	10.50
JH17NV20	110.5	0.028	2.38	0.030	2.52	5.87
VJ02JL10	111.3	0.054	6.81	0.056	7.13	4.70
JO08NV17	113.1	0.035	4.10	0.041	4.77	16.27
VJ08JL20	114.0	0.021	2.77	0.019	2.52	-9.14
JH02AU03	114.6	0.014	1.18	0.013	1.13	-4.70
VJ03JL13	114.8	0.021	2.85	0.028	3.68	29.35
JO02AU03	125.2	0.013	1.53	0.011	1.24	-18.84
JH12NV16	126.0	0.025	2.12	0.024	2.01	-4.99
JO04AU03	128.5	0.009	1.10	0.021	2.41	119.31
JH09SP11	129.7	0.042	3.58	0.048	4.06	13.49
JH21DC13	131.1	0.007	0.58	0.005	0.40	-30.15
JH01JL20	134.2	0.009	0.66	0.015	1.12	71.57
JH20DC13	142.1	0.007	0.61	0.005	0.40	-34.10
VJ01JL10	147.7	0.002	0.21	0.002	0.29	33.68
JS02NV20	149.3	0.017	1.70	0.015	1.56	-8.73
JO07NV17	180.7	0.038	4.38	0.022	2.55	-41.62
JO01JL20	194.1	0.019	2.24	0.023	2.63	17.47
Average	**125.96**	**0.023**	**2.49**	**0.026**	**2.80**	**19.19**

Legend: Data tabulated with file number (column 1) and mean initial ISI (column 2) for 58 motor units. EMG magnitude data from the initial (column 3) and final phases (column 5) of a run, is tabulated along with their respective calculated percent MVC (initial – column 4, final - column 6). Percent change in EMG magnitude is on column 7. Data is tabulated for fast and slow units separately.

Appendix – D

ISI variability data for all 60 motor units

File # Fast units	Initial Mean ISI (ms)	CoVar - Initial	CoVar - Final	Percent change in CoVar
VJ25FE26	66.7	0.109	0.177	61.93
JH27FE7	67.1	0.162	0.241	48.46
VJ23FE7	68.3	0.107	0.163	52.58
VJ18DC13	69.0	0.109	0.148	35.32
VJ13NV17	69.8	0.127	0.243	90.94
CV06OT26	72.3	0.164	0.138	-16.16
JH24DC17	74.8	0.147	0.199	35.03
VJ10OT25	74.9	0.124	0.131	6.07
JH26FE7	76.5	0.134	0.175	30.71
JS06DC17	76.6	0.141	0.176	24.47
VJ09OT25	77.8	0.122	0.263	116.05
JH28FE26	78.4	0.130	0.240	84.62
VJ11OT25	78.5	0.155	0.188	20.97
VJ21FE7	79.7	0.130	0.137	5.79
JS05DC17	81.5	0.157	0.212	35.46
CV08OT26	82.6	0.200	0.167	-16.75
VJ20DC21	82.8	0.127	0.152	19.29
VJ14NV17	83.2	0.122	0.138	13.17
JH19DC13	83.2	0.138	0.176	28.00
JS10FB27	83.4	0.194	0.307	57.99
VJ22FE7	83.5	0.123	0.196	59.35
JH22DC13	83.9	0.134	0.184	37.31
CV11NV16	84.6	0.177	0.138	-21.81
JS04NV20	84.9	0.101	0.148	46.53
JH11OT29	87.5	0.222	0.381	71.62
VJ19DC21	87.7	0.327	0.197	-39.82
VJ15NV17	88.1	0.125	0.143	14.86
JS07FE7	88.3	0.119	0.132	10.97
CV09NV16	89.5	0.218	0.228	4.60
CV01AU03	90.4	0.140	0.214	53.05
CV14NV19	92.0	0.187	0.280	49.87
VJ04JL13	92.0	0.212	0.194	-8.51
JS09FB27	92.4	0.165	0.200	20.91
CV15DC21	93.1	0.160	0.233	46.08
VJ05JL17	95.4	0.141	0.164	15.96
JS11FB27	97.5	0.172	0.210	22.16
JH07SP11	99.4	0.230	0.354	54.03
Average	**82.6**	**0.155**	**0.199**	**31.65**

File # Slow units	Initial Mean ISI (ms)	CoVar - Initial	CoVar - Final	Percent change in CoVar
VJ16NV17	103.3	0.202	0.236	16.87
CV03SP11	104.0	0.272	0.459	68.88
JS08FE7	104.3	0.150	0.237	58.19
VJ06JL17	105.7	0.124	0.159	27.82
JH03AU03	105.8	0.377	0.350	-7.04
JH14NV16	107.3	0.229	0.434	89.93
JH17NV20	110.5	0.215	0.471	119.35
VJ02JL10	111.4	0.094	0.155	64.89
JO08NV17	113.1	0.230	0.288	25.27
VJ08JL20	114.0	0.129	0.212	64.98
JH02AU03	114.6	0.179	0.305	70.87
VJ03JL13	114.9	0.130	0.114	-12.36
JO02AU03	125.2	0.133	0.147	10.53
JH12NV16	126.0	0.189	0.183	-3.44
JO04AU03	128.5	0.273	0.209	-23.30
JH09SP11	129.7	0.231	0.317	37.01
JH21DC13	131.1	0.299	0.383	28.31
JH01JL20	134.2	0.219	0.190	-13.27
JH20DC13	142.2	0.383	0.454	18.54
VJ01JL10	147.7	0.210	0.372	77.14
JS02NV20	149.3	0.194	0.183	-5.43
JO07NV17	180.7	0.365	0.474	29.86
JO01JL20	194.1	0.377	0.341	-9.55
Average	**126.0**	**0.226**	**0.290**	**31.92**

Legend: Data tabulated with file number (column 1) and mean initial ISI (column 2) for 58 motor units. Initial coefficient of variation (column 3) and final coefficient of variation (column 4) are tabulated along with percent change in coefficient of variation (column 5). Data is tabulated for fast and slow units separately.

Appendix – E

MUAP duration data for 56 motor units

File # Fast units	ISI - Mean (ms)	Initial MUAP Duration (s)	Final MUAP Duration (s)	Percent change in MUAP duration
VJ25FE26	66.7	0.0111	0.0168	50.91
JH27FE7	67.1	0.0078	0.0095	22.20
VJ23FE7	68.3	0.0116	0.0120	2.67
VJ18DC13	68.9	0.0090	0.0096	6.56
VJ13NV17	69.8	0.0081	0.0095	17.44
CV06OT26	72.3	0.0095	0.0101	6.38
JH24DC17	74.8	0.0054	0.0072	32.70
JH26FE7	76.5	0.0073	0.0075	3.51
JS06DC17	76.5	0.0080	0.0094	16.43
VJ09OT25	77.8	0.0089	0.0108	21.57
JH28FE26	78.3	0.0075	0.0081	8.28
VJ21FE7	79.6	0.0067	0.0067	1.05
JS05DC17	81.4	0.0095	0.0109	14.48
CV08OT26	82.6	0.0084	0.0092	9.60
VJ20DC21	82.7	0.0121	0.0114	-6.07
JH19DC13	83.2	0.0082	0.0070	-14.44
JS10FB27	83.4	0.0086	0.0077	-10.75
VJ22FE7	83.4	0.0090	0.0100	11.03
CV11NV16	84.5	0.0098	0.0120	22.19
JS04NV20	84.8	0.0095	0.0124	30.85
JH11OT29	87.5	0.0078	0.0070	-9.40
VJ19DC21	87.7	0.0116	0.0145	25.41
VJ15NV17	88.1	0.0043	0.0047	8.40
JS07FE7	88.3	0.0103	0.0095	-7.86
CV09NV16	89.5	0.0078	0.0090	14.85
CV01AU03	90.4	0.0070	0.0076	7.38
CV14NV19	92.0	0.0084	0.0097	16.03
VJ04JL13	92.0	0.0069	0.0079	14.97
JS09FB27	92.4	0.0078	0.0095	22.36
CV15DC21	93.1	0.0087	0.0086	-1.95
VJ05JL17	95.3	0.0062	0.0068	8.67
JS11FB27	97.5	0.0092	0.0094	1.90
JH07SP11	99.4	0.0061	0.0064	4.61
Average	**82.9**	**0.0084**	**0.0093**	**10.7**

File # Slow units	ISI - Mean (ms)	Initial MUAP Duration (s)	Final MUAP Duration (s)	Percent change in MUAP duration
VJ16NV17	103.3	0.0034	0.0042	22.35
CV03SP11	104.0	0.0064	0.0073	14.05
JS08FE7	104.3	0.0097	0.0111	15.30
VJ06JL17	105.6	0.0070	0.0072	2.21
JH03AU03	105.8	0.0050	0.0051	2.07
JH14NV16	107.3	0.0058	0.0071	22.64
JH17NV20	110.5	0.0060	0.0065	9.29
VJ02JL10	111.3	0.0077	0.0087	13.11
JO08NV17	113.1	0.0079	0.0095	19.49
VJ08JL20	114.0	0.0076	0.0084	11.88
JH02AU03	114.6	0.0071	0.0053	-25.33
VJ03JL13	114.8	0.0064	0.0064	-0.03
JO02AU03	125.2	0.0071	0.0074	4.62
JH12NV16	126.0	0.0067	0.0089	32.34
JO04AU03	128.5	0.0050	0.0064	28.72
JH09SP11	129.7	0.0074	0.0066	-10.95
JH21DC13	131.1	0.0059	0.0063	6.16
JH01JL20	134.2	0.0051	0.0061	21.59
JH20DC13	142.1	0.0054	0.0056	4.00
VJ01JL10	147.7	0.0058	0.0059	1.36
JS02NV20	149.3	0.0076	0.0105	39.02
JO07NV17	180.7	0.0075	0.0077	3.22
JO01JL20	194.1	0.0083	0.0090	9.03
Average	**126.0**	**0.0066**	**0.0073**	**10.7**

Legend: Data tabulated with file number (column 1) and mean initial ISI (column 2) for 56 motor units. Initial MUAP duration (column 3) and final MUAP duration (column 4) are tabulated along with percent change in MUAP duration (column 5). Data is tabulated for fast and slow units separately.

Appendix – F

MUAP amplitude data for 56 motor units

File # Fast units	ISI - Mean (ms)	Initial MUAP Amplitude (mV)	Final MUAP Amplitude (mV)	Percent change in MUAP amplitude
VJ25FB26	66.7	0.167	0.160	-4.19
JH27FB7	67.1	0.222	0.369	66.22
VJ23FE7	68.3	0.033	0.065	96.97
VJ18Dc13	68.9	0.063	0.052	-17.46
VJ13Nv17	69.8	0.213	0.150	-29.58
CV06OT26	72.3	0.199	0.165	-17.09
JH24DC17	74.8	0.061	0.072	18.03
JH26FB7	76.5	0.192	0.233	21.35
JS06DC17	76.5	0.209	0.186	-11.00
VJ09OT25	77.8	0.009	0.012	33.33
JH28FB26	78.3	0.174	0.200	14.94
VJ21FB 7	79.6	0.062	0.069	11.29
JS05DC17	81.4	0.214	0.222	3.74
CV08OT26	82.6	0.145	0.158	8.97
VJ20DC21	82.7	0.117	0.119	1.71
JH19DC13	83.2	0.127	0.087	-31.50
JS10FB27	83.4	0.438	0.422	-3.65
VJ22FE7	83.4	0.062	0.077	24.19
CV11Nv16	84.5	0.129	0.168	30.23
JS04Nv20	84.8	0.074	0.059	-20.27
JH11OT29	87.5	0.279	0.297	6.45
VJ19Dc21	87.7	0.048	0.077	60.42
VJ15NV17	88.1	0.065	0.059	-9.23
JS07FE7	88.3	0.087	0.074	-14.94
CV09Nv16	89.5	0.356	0.390	9.55
CV01AU03	90.4	0.187	0.187	0.00
CV14Nv19	92.0	0.383	0.406	6.01
VJ04JL13	92.0	0.109	0.109	0.00
JS09FB27	92.4	0.464	0.407	-12.28
CV15DC21	93.1	0.267	0.278	4.12
VJ05JL17	95.3	0.124	0.119	-4.03
JS11FB27	97.5	0.831	0.984	18.41
JH07SP11	99.4	0.072	0.088	22.22
Average	**82.9**	**0.187**	**0.198**	**8.57**

File # Slow units	ISI - Mean (ms)	Initial MUAP Amplitude (mV)	Final MUAP Amplitude (mV)	Percent change in MUAP amplitude
VJ16Nv17	103.3	0.044	0.042	-4.55
CV03SP11	104.0	0.053	0.052	-1.89
JS08FE7	104.3	0.267	0.283	5.99
VJ06JL17	105.6	0.071	0.074	4.23
JH03AU3	105.8	0.104	0.104	0.00
JH14NV16	107.3	0.182	0.194	6.59
JH17NV20	110.5	0.134	0.140	4.48
VJ02JL10	111.3	0.068	0.064	-5.88
JO08NV17	113.1	0.130	0.145	11.54
VJ08JL20	114.0	0.127	0.129	1.57
JH02AU3	114.6	0.065	0.067	3.08
VJ03JL13	114.8	0.073	0.072	-1.37
JO02Au03	125.2	0.046	0.038	-17.39
JH12NV16	126.0	0.091	0.084	-7.69
JO04Au03	128.5	0.036	0.048	33.33
JH09SP11	129.7	0.177	0.193	9.04
JH21DC13	131.1	0.037	0.035	-5.41
JH01JL20	134.2	0.040	0.050	25.00
JH20DC13	142.1	0.064	0.067	4.69
VJ01JL10	147.7	0.016	0.015	-6.25
JS02Nv20	149.3	0.038	0.037	-2.63
JO07Nv17	180.7	0.298	0.305	2.35
JO01Jl20	194.1	0.053	0.049	-7.55
Average	**126.0**	**0.096**	**0.099**	**2.23**

Legend: Data tabulated with file number (column 1) and mean initial ISI (column 2) for 56 motor units. Initial MUAP amplitude (column 3) and final MUAP amplitude (column 4) are tabulated along with percent change in MUAP amplitude (column 5). Data is tabulated for fast and slow units separately.

Appendix – G

MPF data for all 60 motor units

File # Fast units	ISI - Mean (ms)	Initial MPF (Hz)	Final MPF (Hz)	Percent change in MPF
VJ25FE26	66.7	87.32	72.97	19.67
JH27FE7	67.1	137.26	113.59	20.84
VJ23FE7	68.3	111.03	101.83	9.04
VJ18DC13	68.9	125.50	111.92	12.12
VJ13NV17	69.8	106.44	99.65	6.82
CV06OT26	72.3	106.80	96.62	10.53
JH24DC17	74.8	173.52	164.07	5.76
VJ10OT25	74.8	129.98	120.35	8.00
JH26FE7	76.5	132.94	129.61	2.57
JS06DC17	76.5	109.86	99.68	10.21
VJ09OT25	77.8	116.36	105.52	10.27
JH28FE26	78.3	141.59	137.00	3.35
VJ11OT25	78.5	117.04	113.06	3.52
VJ21FE7	79.6	116.11	106.76	8.76
JS05DC17	81.4	90.81	89.48	1.49
CV08OT26	82.6	115.32	101.16	14.01
VJ20DC21	82.7	111.22	93.55	18.89
VJ14NV17	83.1	147.13	137.05	7.35
JH19DC13	83.2	151.13	143.04	5.66
JS10FB27	83.4	108.49	101.74	6.63
VJ22FE7	83.4	112.80	108.41	4.05
JH22DC13	83.9	156.65	141.05	11.06
CV11NV16	84.5	100.86	86.03	17.24
JS04NV20	84.8	127.53	122.07	4.47
JH11OT29	87.5	157.18	149.19	5.36
VJ19DC21	87.7	114.59	101.73	12.65
VJ15NV17	88.1	152.14	140.38	8.37
JS07FE7	88.3	114.43	109.33	4.67
CV09NV16	89.5	107.26	95.63	12.16
CV01AU03	90.4	132.34	125.88	5.14
CV14NV19	92.0	107.96	92.84	16.28
VJ04JL13	92.0	143.58	123.96	15.83
JS09FB27	92.4	114.42	108.51	5.45
CV15DC21	93.1	96.95	96.96	0.00
VJ05JL17	95.3	148.19	136.66	8.43
JS11FB27	97.5	105.87	104.26	1.55
JH07SP11	99.4	150.30	140.76	6.77
Average	**82.6**	**123.75**	**114.12**	**8.78**

File # Fast units	ISI - Mean (ms)	Initial MPF (Hz)	Final MPF (Hz)	Percent change in MPF
VJ16NV17	103.3	147.11	133.57	10.14
CV03SP11	104.0	166.60	157.11	6.04
JS08FE7	104.3	104.21	100.05	4.16
VJ06JL17	105.6	141.07	136.23	3.55
JH03AU03	105.8	175.45	176.71	-0.71
JH14NV16	107.3	147.52	140.16	5.25
JH17NV20	110.5	149.11	138.32	7.80
VJ02JL10	111.3	131.03	122.74	6.75
JO08NV17	113.1	124.11	119.36	3.98
VJ08JL20	114.0	136.76	139.88	-2.23
JH02AU03	114.6	165.95	165.91	0.02
VJ03JL13	114.8	159.52	150.02	6.33
JO02AU03	125.2	150.00	155.75	-3.69
JH12NV16	126.0	148.07	144.01	2.82
JO04AU03	128.5	143.64	153.90	-6.67
JH09SP11	129.7	140.20	133.33	5.15
JH21DC13	131.1	148.58	160.73	-7.56
JH01JL20	134.2	141.17	136.65	3.31
JH20DC13	142.1	142.78	160.62	-11.11
VJ01JL10	147.7	131.10	109.51	19.71
JS02NV20	149.3	132.92	137.47	-3.30
JO07NV17	180.7	134.11	126.77	5.79
JO01JL20	194.1	150.08	168.55	-10.96
Average	**126.0**	**143.96**	**142.06**	**1.94**

Legend: Data tabulated with file number (column 1) and mean initial ISI (column 2) for all 60 motor units. Initial MPF (column 3) and final MPF (column 4) are tabulated along with percent change in MPF (column 5). Data is tabulated for fast and slow units separately.

Appendix – H

AC force data for 29 motor units

File # Fast units	ISI - Mean (ms)	SD of AC Force Initial (g)	SD of AC force final (g)	Percent change in SD of AC force
VJ25FE26	66.7	8.15	24.94	205.89
JH27FE7	67.1	42.72	165.57	287.56
VJ23FE7	68.3	14.48	44.67	208.52
JH26FE7	76.5	51.33	72.09	40.43
JH28FE26	78.3	3.36	7.71	129.36
VJ21FE7	79.6	17.87	27.31	52.81
JS10FB27	83.4	6.56	10.33	57.61
VJ22FE7	83.4	15.90	31.75	99.70
CV11NV16	84.5	2.95	8.23	178.96
JS04NV20	84.8	5.47	6.83	24.83
JH11OT29	87.5	9.14	37.27	307.72
JS07FE7	88.3	49.09	51.29	4.50
CV09NV16	89.5	6.35	7.25	14.22
CV01AU03	90.4	12.26	12.73	3.81
CV14NV19	92.0	4.19	9.14	117.90
JS09FB27	92.4	4.21	5.62	33.39
JS11FB27	97.5	8.70	13.09	50.42
JH07SP11	99.4	4.44	7.57	70.24
Average	**83.9**	**14.84**	**30.19**	**104.88**
Slow units				
VJ16NV17	103.3	2.76	2.92	5.97
CV03SP11	104.0	7.76	11.96	54.01
JS08FE7	104.3	41.96	58.78	40.10
JH03AU03	105.8	3.06	3.64	18.98
JH14NV16	107.3	2.57	3.78	46.91
JH17NV20	110.5	3.28	4.44	35.23
JO08NV17	113.1	5.67	5.94	4.78
JO04AU03	129.5	6.89	9.88	43.54
JH09SP11	129.7	13.77	24.21	75.85
JS02NV20	149.3	4.13	6.74	63.12
JO07NV17	180.7	4.60	4.13	-10.20
Average	**121.6**	**8.77**	**12.40**	**34.39**

Legend: Data tabulated with file number (col. 1), mean initial ISI (col. 2) for 29 motor units, initial (col. 3) and final SD of AC Force (col. 4) are tabulated along with percent change in SD of AC force (col. 5). Data is tabulated for fast and slow units separately.

Appendix – I

Slope data for all motor unit pairs (targeted and non-targeted units)

Category 1			
Targeted Unit file #	**Targeted Unit slope**	**Non-targeted Unit file #**	**Non-targeted Unit slope**
CV03SP11	-0.0018	CV34	0.0112
JH17NV20	0.0006	JH1718NV20	-0.0248
JH03AU03	0.0020	JH04AU03	-0.0621
JH07SP11	-0.0006	JH78SP11	0.1284
JH12NV16	0.0023	JH12,13NV16	0.0078
JH14NV16	-0.0040	JH14,15NV16	0.0033
JH24DC17	0.0010	JH24,25DC	-0.0034
JO02AU03	0.0138	JO03AU03	0.0141
JS02NV20	0.0002	JS02,03NV20	-0.0037
Category 2			
Targeted Unit file #	**Targeted Unit slope**	**Non-targeted Unit file #**	**Non-targeted Unit slope**
JH03AU03	0.0006	JH05AU03	-0.0036
JH03AU03	-0.0001	JH06AU03	0.0008
CV01AU03	0.0021	CV02AU03	0.0028
CV08OT26	0.0287	CV26OT87	0.0252
CV11NV16	0.0004	CVNV1112	-0.1514
VJ06JL17	0.0009	VJ07JL17	-0.0026
JO04AU03	0.0023	JO05AU03	-0.0050

Legend: Data tabulated with targeted (column 1) and non-targeted unit file numbers (column 2) and their respective slope data values (targeted unit – column 2, non-targeted unit – column 4). Data is tabulated for category 1 and 2 separately.

Appendix – J

Coefficient of variation data for all motor unit pairs (targeted and non-targeted units)

Category 1							
Targeted unit file #	**Initial CoVar**	**Final CoVar**	**Percent change in CoVar**	**Non-targeted unit file #**	**Initial CoVar**	**Final CoVar**	**Percent change in CoVar**
CV03SP11	0.280	0.477	70.38	CV34	0.226	0.240	5.91
JH17NV20	0.298	0.507	70.22	JH1718NV20	0.210	0.187	-10.67
JH03AU03	0.484	0.383	-20.86	JH04AU03	0.373	0.320	-14.25
JH07SP11	0.195	0.283	44.86	JH78SP11	0.223	0.281	26.22
JH12NV16	0.205	0.140	-31.82	JH12,13NV16	0.159	0.183	14.99
JH14NV16	0.248	0.409	65.26	JH14,15NV16	0.270	0.250	-7.59
JH24DC17	0.152	0.183	20.59	JH24,25DC	0.231	0.515	122.38
JO02AU03	0.148	0.525	253.67	JO03AU03	0.331	0.227	-31.37
JS02NV20	0.266	0.236	-11.58	JS02,03NV20	0.190	0.263	38.46
Category 2							
Targeted unit file #	**Initial CoVar**	**Final CoVar**	**Percent change in CoVar**	**Non-targeted unit file #**	**Initial CoVar**	**Final CoVar**	**Percent change in CoVar**
JH03AU03	0.526	0.383	-27.30	JH05AU03	0.477	0.312	-34.70
JH03AU03	0.503	0.400	-20.45	JH06AU03	0.454	0.272	-40.23
CV01AU03	0.224	0.294	31.35	CV02AU03	0.447	0.552	23.53
CV08OT26	0.171	0.287	68.03	CV26OT87	0.297	0.257	-13.70
CV11NV16	0.114	0.134	16.80	CVNV1112	0.252	0.270	7.07
VJ06JL17	0.118	0.148	25.69	VJ07JL17	0.250	0.272	8.88
JO04AU03	0.189	0.162	-14.20	JO05AU03	0.255	0.241	-5.64

Legend: Data tabulated with targeted (column 1) and non-targeted unit file numbers (column 5). The initial and final coefficient of variation (CoVar) data values and their corresponding percent change in CoVar over time. Data is tabulated for category 1 and 2 separately.