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THE ROLE OF THE NEUROMUSCULAR JUNCTION IN EXERCISE-INDUCED FATIGUE, AS INVESTIGATED BY THE USE OF A CHOLINESTERASE INHIBITOR.

by

Gregory James Jensen

B.Sc.(Hons.), Physiology, University of British Columbia, 1985

THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE (KINESIOLOGY)

In The School Of Kinesiology

Gregory James Jensen 1988

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December 1988

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7/12/88
Transmission failure during repetitive muscular activity has been a topic for investigation since the early 1950's. While it can be readily demonstrated that transmission failure is present under certain conditions in animal preparations, it has yet to be proven a limiting factor in human muscular performance. The present study consisted of two experiments designed to test the integrity of neuromuscular transmission in humans performing maximal exercise of the quadriceps muscle. It was predicted that interavenous administration of edrophonium chloride (a cholinesterase inhibitor) would have an effect on the normal time course of fatigue by (i) extending the time to onset of fatigue, TTO (defined as the point when force output fell to 90% of the maximal voluntary contraction, MVC) and/or (ii) extending the time to pre-defined fatigue levels, TTF (55% MVC for the dynamic trials and 60% MVC for the static trials). As well, the force output at a given time during fatigue, defined as the time-specific MVC (TS%MVC), was investigated and predicted to increase with edrophonium chloride. It was also postulated that if transmission failure was present, whether correlated to force loss or not, the integrated electromyography (IEMG) would be quantitatively higher after edrophonium chloride.

In Experiment 1 subjects performed both static and dynamic exercise two minutes after receiving an injection of 10mg edrophonium chloride and 0.3mg of atropine, or the control (0.03mg atropine and normal saline). The results revealed that edrophonium chloride had no effect on any of the force variables described above, for both static and dynamic-induced fatigue. Furthermore, there were no significant changes in IEMG with the drug. These observations reject the hypothesis that transmission failure is a mechanism of force loss during maximal exercise in muscles capable of maximal activation.

Experiment 2 was conducted to investigate the effects of edrophonium chloride under conditions where the quadriceps was unable to produce maximal activation. This was accomplished by having the subject produce 40 maximal dynamic contractions prior to drug injection. After a one minute absorption period the subjects exercised both statically and dynamically as in Experiment 1. During these trials, the pre-fatigue
quadriceps produced peak tensions that were 25% and 15% (dynamic and static respectively) lower than the values obtained from Experiment one. Edrophonium chloride was unable to restore the peak tensions to values comparable to Experiment 1, thus ruling out transmission failure as the source of this force loss. Furthermore, consistent with Experiment 1, edrophonium chloride did not have an effect on the force variables defined in Experiment 1, nor were there any significant differences in the peak tensions or the IEMG.

The conclusions derived from both experiments suggest that during short term, high-intensity exercise of the quadriceps, failure of electrical propagation and neuromuscular transmission is an unlikely mechanism of force loss during skeletal muscle fatigue.
This thesis is dedicated to all members of the Jensen family, who all, in some way, have contributed to the successful completion of this master degree. In particular, my brother, nicknamed "cheeze", should receive an equal degree, for without his support over the years, my success would have been difficult. Our mother, however, deserves the greatest applause, since it is from her silent teachings that success and happiness have followed her children. This thesis represents success as measured by the academic institution, in turn the following words, arranged by Ralph Waldo Emerson (1803-1882), best describes what I have learned about success as measured by my mother:

"To laugh often and love much; to win the respect of intelligent persons and the affection of children; to earn the approbation of honest critics and to endure the betrayal of false friends; to appreciate beauty; to find the best in others; to give of one's self; to leave the world a bit better, whether by a healthy child, a garden patch, or a redeemed social condition; to have played and laughed with enthusiasm and sung with exultation; to know that even one life has breathed easier because you have lived—this is to be successful."
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PART A
INTRODUCTION AND LITERATURE REVIEW
0.1 Introduction

To produce a single movement, whether it be voluntary or reflexive, requires the interaction of a number of physiological processes within and beyond the motor unit. These processes must function within the biochemical, electrical, and mechanical limits of the neuromuscular system to provide optimal muscle performance. During sustained muscular contraction, however, failure of one or more of these processes promotes the symptoms of muscular fatigue, including a significant reduction in physical performance during work or play. Skeletal muscle fatigue has long been a topic of investigation. Defined as the inability of the neuromuscular system to maintain sufficient force for a given activity, the course of muscle fatigue can vary significantly between individuals. This has made the study of fatigue difficult and left science with no universal agreement as to a major limiting mechanism.

Over the past few decades, our knowledge about the neuromuscular system has greatly expanded. This has permitted researchers confidently to dismiss the idea that a single mechanism limits optimal muscle performance, and to pursue a multicomponent model (Green, 1988). Construction of a model has resulted in a greater understanding of the events that lead to muscular contraction, which has allowed easier prediction of the sites most likely to fail. Figure 1 illustrates a simplified sketch of the neuromuscular pathway for a voluntary muscular contraction. The cognitive events that initiate a voluntary contraction originate in the motor cortex of the brain. Before the signal is sent to the periphery it is thought to undergo modification, traveling through various association and "fine tuning" areas of the CNS, of which the details are highly unresolved. Propagation of the signal down the spinal cord may be direct or via spinal interneurons on route to synapsing in motor neuron (MN) pools of the ventral horns. An action
Figure 1. Schematic representation of the neuromuscular system. (adapted from Bigland-Ritchie, 1981, and Guyton 1982).
potential will be initiated in selected MNs and propagate peripherally where at the distal portion of the MN axon the signal takes several paths depending on the degree of axonal bifurcation. The action potential then terminates at the NMJ facilitating exocytosis of stored acetylcholine (Ach). Subsequent diffusion and reversible binding of Ach to the post-synaptic membrane results in end plate potentials, which if being of threshold amplitude will initiate a muscle action potential (MAP). The MAP spreads along the surface of the sarcolemma and cross-sectional through the transverse tubules (T tubules). Depolarization of the T tubules mediates calcium release from adjacent sarcoplasmic reticulum (SR) cisternae. The increased cytoplasmic calcium releases tropomyosin inhibition by binding to the troponin C complex of the actin filament resulting in actomyosin interaction and muscular contraction. While this outline omits many details it does suggests a number of possible failure sites that could limit optimal muscle performance.

Advancements in technology have permitted a tremendous increase in research into the neuromuscular system, in particular, since the advent of electromyography (EMG). This technique takes advantage of the electrical properties that accompany nerve and muscle cell activity. Sodium and potassium ions are maintained such that sodium is more concentrated on the outside of the cell and potassium on the inside. This distribution develops an electrochemical gradient across the sarcolemma that leaves the inside of a quiescent muscle fibre about 90 mV more electronegative than the outside. During rapid depolarization of the sarcolemma the changing dipole moment can be recorded with electrodes that are inserted into the muscle or placed on the overlying skin. Intracellular needle electrodes are widely used in animal preparations, while noninvasive recording techniques are generally used in human studies. The advantage of using
intracellular electrodes is that the electrical activity of individual motor units can be studied, as where surface electrodes can only provide an index of the electrical activity of a group of motor units. Researchers who employ the surface EMG technique are presented with several possible sources of error or ambiguity which may affect data interpretation. The EMG signal will vary greatly between individuals, as well as within subjects during repeated measures, if the electrodes are not carefully placed and the underlying skin is not properly prepared. The electrodes pick up activity directly below, which includes not only the muscle of interest, but also electrical activity from adjacent synergists. If electrode placement is not consistent, contaminating electrical activity may be amplified or attenuated, which could significantly affect the results. Also areas of high subcutaneous fat yields high electrical impedance, thus adding another consideration when choosing an electrode site (Rau 1985, and Lippond, 1967). The greatest problem encountered, however, when interpreting EMG data, is accounting for the activities of the individual motor unit. It is not fully understood how different motor units respond to variable demands from the CNS, and how the individual changes are reflected in the EMG signal.

0.2. The Motor Unit

The motor unit, which consists a few to several hundred muscle fibres innervated by a single multi-branched alpha motor neuron, has been classified in a number of different ways based on the mechanical, biochemical, and electrical properties of the MN (Burke, 1981).

The development of systems of classification for both motor units and muscle fibre types has occurred in parallel with advancements in technology. Ranvier’s simple classification, proposed in the late 1800’s, which linked
contraction time to the color of the muscle tissue (red or white), has been revised many times resulting in classifications that depend upon complex biochemical and histochemical analyses. For the purpose of this paper the classification for muscle fibres of Peter et al (1972) will be used, however, when referring to the motor unit Burke’s (1981) nomenclature will be used.

The first group of muscle fibres have been termed slow oxidative fibres (SO, or sometimes called type I fibres or, as an intact motor unit, S-type). These are small fibres, with slow contraction times, that generate relatively low peak tensions, but are essentially fatigue resistant. The second group of muscle fibres are the fast twitch or type II fibres. Under this heading three subgroups have been defined: (i) Fast twitch oxidative-glycolytic fibres (FOG, type IIA, or as a motor unit, FR-type), which are similar to the SO type due to their fatigue resistant-oxidative potential, but are capable of generating much greater forces. (ii) Fast twitch glycolytic fibres (FG, IIB, or FF-type) are the largest muscle fibres and are usually required only for quick, powerful contractions because of their high force generating capacity and low fatigue resistance. The third fibre type under the fast twitch heading are the Intermediate fast twitch fibres (IIAB or F[inter]), and as the name suggests, these fibres have properties inbetween FG and FOG fibres.

Although this classification system segregates motor units into distinct groups, the fact that IIAB fibres have been defined suggests that there is probably a continuous spectrum of fibres ranging from SO to FG characteristics (Kugelberg, 1979). The observation that muscles of predominantly SO or FG composition demonstrate smooth force and fatigue curves supports this concept. There is also evidence that shows that under certain conditions SO fibres can adopt the characteristics of FG fibres and vise versa (Edstrom and Grimby, 1986).
The type of motor unit activated will determine the appearance of the surface EMG signal since an action potential from a fiber of twice the diameter may be four times greater than that of the smaller unit (Loeb and Gans, 1986). The SO fibres are smaller in diameter, are less numerous per motor unit, and have different contractile properties than the FG type. Thus, in order to produce the muscle contraction for a desired movement, the neuromuscular system must strategically recruit motor units in a specific pattern. It has been generally accepted that, during most movements, motor units are recruited according to the size principle, as proposed by Henneman (1965) (although, under certain contractile demands this theory has been refuted [Edström and Grimby, 1986]) For activities that demand smaller forces for long periods of time, SO and FOG type are recruited first followed by FG fibers if the force requirements increase or fatigue develops. A plot of EMG vs. force demonstrates both a linear and non-linear positive relationship as illustrated in Figure 2. The linear response of the soleus and the non-linear response of the biceps brachii have been attributed to the heterogeneous fiber composition of the latter and the homogeneous composition of the former (Bigland-Ritchie, 1981 and Pagala, 1984). These relationships hold under optimal conditions, but can change during fatigue, when motor units tend to be activated synchronously providing better efficiency at the expense of a smooth pattern of force generation (Rau, 1985). The relationships may also vary when motor units of a common type are subjected to stimuli that promotes the adoption of the characteristics of another fiber type (Edstrom and Grimby, 1986).

In order to confidently apply surface EMG to the study of muscle function a greater understanding of the roles of the different motor units is needed. The remainder of this review will concentrate on mechanisms that have been postulated to regulate the course of skeletal muscle fatigue.
postulated to regulate the course of skeletal muscle fatigue.

The sites of muscle fatigue can be divided into two groups: (i) Those processes that originate proximal to the motor unit termed central fatigue, and (ii) those sites that are peripheral in origin and include all processes that involve motor unit activity.

0.3 Central Fatigue

The ability of the CNS to maintain its drive to an active motor unit has been questioned for as long as research into muscle fatigue has been conducted. Unfortunately progress in this area has been limited because of the complex nature of the CNS and the lack of technology developed to study it. One of the

Figure 2. EMG/froce relationship for unfatigued soleus and biceps muscle. (Ref. Bigland-Ritchie, 1981).
observation that an individual could produce more work shortly after mental arousal (in this case the mental arousal was an important lecture given by a Mosso colleague) than before suggested to Mosso that fatigue must be primarily central. Several years later Asmunssen, also interested in central fatigue, set up a series of experiments to test Mosso’s hypothesis. Using a simple arm ergograph, Asmunssen had subjects produce a series of maximal contractions until their force fell to zero. During a subsequent two minute rest period one group of subjects sat still doing nothing, while a second group of subjects spent the rest period performing some “diverting” activity (ie. reading). Following the rest period, all of the subjects conducted a second series of contractions on the ergograph. It was shown that more work was done by the subjects who performed diverting activity during the rest period than those who sat idle. Similar conclusions were extracted in a separate set of experiments where Asmunssen had subjects exercise with their eyes closed followed by an immediate open eyed bout, and vise versa. In the condition where the subjects eyes were first closed then opened, 20-30% more work was performed compared to if the procedures were reversed. Like Mosso, Asmunssen interpreted these results to suggest that the state of arousal of the CNS plays a major role in the fatigueability of the neuromuscular system.

Although the observations of Mosso and Asmunssen are interesting and provide clues to a possible source of fatigue, a more rigid definition and mechanistic explanation is needed to link a central element to muscle fatigue. In more recent publications (Bigland-Ritchie, 1981) central fatigue has been distinguished from peripheral with the use of superimposed supramaximal nerve stimulation. The concept is quite simple; if the loss in force of a given muscle, that has been voluntarily activated, can be restored, or partially restored by a
supramaximal stimulation of the innervating nerve, then the recovered force must have a central origin (or at least an origin proximal to the point of stimulation). It also follows that the unrestored force loss must therefore be peripheral, or originate somewhere distal to the point of stimulation.

Comparisons of forces generated by volition and nerve stimulation have been done several times. It was originally thought that maximal tension was not attainable through voluntary effort. This idea has been rejected since it has now been shown that most human muscle can be voluntarily activated to match the tension induced by maximal nerve stimulation (Belanger and McComas, 1981, and Bigland-Ritchie, 1979)

Data from experiments comparing fatigue curves from voluntary effort and maximal nerve stimulation has been confusing. Some studies (Grimby, 1981, and Bigland-Ritchie, 1979) have shown that voluntary force falls faster than force induced by maximal nerve stimulation. This would suggest that there may be some degree of failure of the motor drive to the muscle. However, it is difficult to separate whether this observation is due to central mechanisms or simply a consequence of the stimulation protocol, since others (Bellemare and Garzaniti, 1988) have demonstrated no difference or the opposite of the above findings. A study by Bigland-Ritchie (1979) lead to the conclusion that failure of central mechanisms was at least partly responsible for the loss of force observed during MVC of human quadriceps muscle. In this study Bigland-Ritchie observed that three of the nine subjects were able to restore some of the lost force if they were encouraged to apply an "extra effort". At the points of "extra effort" both the EMG and force output showed a transient increase, with the increase in EMG being quantitatively higher. Furthermore, the subjects who demonstrated the "extra effort" also showed a more rapid decline in force output during voluntary
effort than from stimulation of the femoral nerve. Based on these observations, Bigland-Ritchie concluded that the three subjects who produced "extra efforts" showed signs of central fatigue. A closer look at the data, however, reveals several arguments against their conclusions. Firstly, the paper supplied sample data from one of the "centrally" fatigueing subjects and one of the other six non-centrally fatigueing subjects. The peak tension from the former subject appeared significantly lower than that of the latter subject, and during the bouts of "extra effort" the restored force produced was, at a few points, greater than the initial values. This suggests that the three subjects were not suffering from failure of central drive, but rather from lack of experience in producing maximal forces. This is supported by Bigland-Ritchie in a later publication (1984) where she states that maximal voluntary contraction can only be sustained by well motivated, trained subjects. The subjects in the 1979 study were not trained. Grimby (1981), who originally found the same "extra effort" patterns in a similar study, trained a second group of subjects and found that as the trained level increased the ability to produce "extra efforts" declined. This, and the appearance of a longer duration of the EMG plateau phase with the "centrally" fatigueing subjects suggests initial failure of maximal activation rather than a central fatigueing process.

Although the evidence in these and earlier studies were not conclusive in demonstrating central failure, or for that matter a mechanism by which central failure occurs, central precursors to fatigue can not be rejected. In fact, a number of possibilities have recently been postulated. The motor neurons receive an extensive network of excitatory and inhibitory inputs from both the CNS and the periphery (Brooks, 1986, Garland et al., 1988, Suzuki et al., 1988). Afferent feedback can originate from a number of peripheral receptors, which may include,
pain, temperature, muscle tension and length, and feedback from the chemical
environment. Signals from some of these variables are feedback to the CNS and
motor neuron pools where they theoretically and in some cases experimentally
have been shown to influence motor unit output (Enoka, 1985; Brooks, 1986;
Green, 1987; Asmussen, 1979; Garland, Garner, and McComas, 1988).

The feedback systems are particularly influential on the perception of effort,
which is important in maintaining MVC, as demonstrated by Grimby (1981) and
Bigland-Ritchie (1979). Subjects that receive visual feedback of force during
fatigueing exercise protocols are able sustain higher contractions for longer,
suggesting that their perception of effort is distorted by feedback from
non-visual information.

There is some evidence that corollary signals form the efferent signal in
the higher brain centers are relayed to the cerebellum and red nucleus where
they may interface with afferent feedback from the periphery, and final efferent
outputs will be modulated accordingly (Brooks, 1986, Enoka, 1985, and Mathews,
1977). Evidence to support modulation of the efferent signal from the information
extracted from the working muscle has been provided in tendon vibration reflex
studies (McClosky, 1987). Vibrating a muscle tendon at a high frequency (>100Hz),
selectively activates the la afferents eliciting a spinal reflex to the alpha motor
neurons. If a subject supports a weight with the biceps at a constant joint angle,
over time fatigue will set in and the weight will feel increasingly more difficult
to support. Application of high frequency vibration to the agonist tendon induces
a spinal reflex facilitation, and the subject becomes less aware of fatigue and
supports the weight longer. By moving the vibration to the antagonist tendon
(triceps) an inhibitory signal is relayed to the biceps alpha motor neuron.
Consequently, to support the weight the efferent signal from the brain must be
greater. In this case the perception of effort by the subject is greater and the weight is not supported as long (McClosky, 1978). The mechanism suggested here is that the efferent signal from the higher brain centers is continually sampled to give the individual a perception of what is being sent to the periphery as well as a mechanism of comparing this signal with incoming information from the muscles. Metabolic by-products of muscular activity that are capable of crossing the blood brain barrier may also effect the central drive. Such substances may include H+ ions or NH3, as suggested by Mutch and Banister (1983).

Although these and the studies discussed earlier are not conclusive in demonstrating a mechanism by which the CNS fatigues, if in fact it does, there is certainly enough evidence that merits further research. However, progress on this topic will depend on a greater understanding of the CNS circuitry, which can only be facilitated with significant technical advancements and the development of a good animal model.

0.4 Peripheral Fatigue

Peripheral inhibition in the neuromuscular system has attracted the most research interest, with the bulk of the research concentrated on contractile failure. Fatigue related to this topic involve the metabolic, mechanical, and biochemical events occurring within an active muscle distal to the endplate. All of the events proximal to the sarcolemma up to and including the motor axon are collectively termed transmission processes, and are the major topic of this paper to be discussed in detail later.
0.4.1 Contractile failure

The metabolic consequences of muscular activity that have been correlated to muscular fatigue include; (i) changes in the energy status of the cell, (ii) electrolyte shifts, (iii) accumulation of contractile by-products, and (iv) structural alterations of the fibre.

Energy from the hydrolysis of ATP is required for operations of the excitation-contraction process, as indicated by the large number of ATPases present throughout the muscle cell. Consequently, it follows that depletion of energy stores during muscular activity could significantly inhibit force production. Measurement of [ATP], [phosphocreatin], and [glycogen], do not correlate well with high intensity fatigue, it is therefore agreed that, at least during short term maximal exercise, that energy depletion is not a primary source of force loss (Dawson, 1987, Salhin, 1983, Hermansen, 1981). ATP utilization appears to be the more important consideration during fatigue. Accompanying the generation of force is also the liberation of cellular by-products, including increased levels of; H+ ions, ADP, phosphates, and lactate, all of which correlate well with fatigue (Dawson, 1978, and Hermansen, 1981). It is still debated as to whether these by-products directly inhibit force generation, or are just a consequence of muscle cell activity. Increased levels of lactate are readily demonstrated both in the blood and intracellularly during fatigue. The residing hypothesis is that the apparent associated H+ ion of lactate (the source of the H+ ion during muscle cell acidosis is not in agreement) inhibits ATPases important in cross-bridging and the subsequent relaxation (Donaldson, 1983, and Duchateau, 1987). Under normal resting conditions the intracellular pH is maintained at about 7.0 with the help of intracellular buffering systems (Tibbits, 1987, Hermansen, Osnes, 1972, and
Sahlin, 1983) and the Na\(^+\) - H\(^+\) exchanger. During exercise, the intracellular pH has been estimated to fall to 6.4-6.3. This reduction in pH inhibits the activity of ATPases responsible for cross-bridging during force generation, as well as inhibiting Ca\(^{++}\) regulation important for both contraction and relaxation (Sahlin, 1983 and Donaldson, 1983). The relaxation of the muscle is primarily a function of the intracellular [Ca\(^{++}\)] which is determined by the rate of release and reuptake of Ca\(^{++}\) by the SR (Belcastro, 1985, Tibbits, 1987; Green, 1987, and Dawson, 1978, 83). At a lower pH during exhaustive exercise Ca\(^{++}\) accumulation in isolated SR preparations was depressed (Belcastro, 1985). There appears to be reasonable agreement that acidosis is related to the fatiguing process, particularly with respect to enzyme activity, but there are still some reservations as to the source of the H\(^+\) ion and its exact role in fatigue.

High frequency stimulation of skeletal muscle has been shown to induce considerable shifts in Na\(^+\) and K\(^+\) down their electrochemical gradients (Vollestad, 1987). These shifts have been hypothesized to leave the sarcolemma less responsive to electrical stimulation resulting in a reduced action potential amplitude (Jones, 1981 and Luttgau, 1965, Juel, 1988). If this is the case, other intracellular membrane structures, such as the T-tubules and SR, may also be affected, which would theoretically upset the excitation-contraction coupling of the fibre (Tibbits, 1987, and Vollestad, 1987).

A final concern in the area of contractile failure is whether or not structural changes in the muscle during exercise occurs. To test for morphological abnormalities, muscles' biopsies have been used (Sjostrom and Friden, 1983). Data from short term high intensity exercise, or from exercise under ischemic conditions appears to induce Z-band disorganization (Sjostrom and Friden, 1983, and Green, 1987), however it is difficult to demonstrate if this has any
correlation to force generation, or if the structural damage is simply a consequence of the biopsies.

Since the primary topic of this paper is transmission failure, proper justice could not be paid to the vast amount of research on contractile fatigue. For more information on this topic excellent reviews have been presented by Green 1987, Vollestad 1988 and selected papers in Biochemical symposium (1983).

0.4.2 Transmission Failure

Although the bulk of research into muscle fatigue has been concentrated at the muscle cell level, metabolic changes have been unsatisfactory in providing a complete explanation. Researchers thus shifted some their interests to more proximal areas of the neuromuscular system. We have already dealt with the CNS and discovered insufficient objective evidence to support a central fatigueing mechanism. The final area to consider is the link between the CNS and the muscle cell. The motor unit axon provides the final path for electrical propagation of the efferent signal to muscle. Transmission of this signal is dependant on a series of complex electrical and biochemical events that occur at the NMJ. Failure of transmission mechanisms became a major topic of investigation in the 1950's subsequent to the classic works of Asmussen (1934) and of Brown and Burns (1949).

The results of Asmussen's (cit. Asmussen, 1979) study are schematically reproduced in figure 3 below. Using a lizard intercostal nerve–muscle preparation, Asmussen observed force changes in response to both direct (muscle) and
(nerve) stimulation. Maximal tetanic stimulation was first applied to the nerve and maintained until the force output fell to zero. At that point the stimulus was immediately switched to the muscle fibre, at which time a significant restoration of force was observed (figure 3). The obvious conclusion of the day was that somewhere between the point of nerve stimulation and the muscle fibre, failure must of occurred that would account for the restored force seen during direct stimulation. The NMJ seemed the most plausible site. Brown and Burns (1949) conducted a similar but more comprehensive investigation testing Assmunsens findings. Using the cat soleus and tibialis anterior muscles they first made

Figure 3. Re-drawn results of Assmunsens original observations of force response to indirect and direct stimulation. (Ref. Assmunsen, 1979)
they first made comparisons between direct and indirect stimulation. These tests revealed that with consistent voltage and stimulation frequency peak tensions were achieved within 5%. However, they reported that the decline in force was generally faster via nerve stimulation. The latter observation was suggestive of neuromuscular block. The preparations were then fatigued both indirectly and directly. At five second intervals along each of the fatigue curves the stimulation was quickly switched from indirect to direct, which showed the same enhancement in force as Asmussen, and from direct to indirect, which resulted in a decline in force. From these results Brown and Burns concluded that neuromuscular block regularly occurs during nerve stimulation, but confused the issue by stating that this block in no way was related to the force loss in fatigue. Their data provide evidence to suggest the former conclusion, however, they did not provide an adequate information to explain the uncoupling of neuromuscular transmission from force.

Before discussing the research that followed from these studies, some important characteristics of the neuromuscular junction will be reviewed.

0.5 The Neuromuscular Junction

The function of the NMJ is to transfer the propagated action potential of the motor nerve axon to the associated skeletal muscle fibre. Morphologically the NMJ can be divided into two components: (i) the presynaptic terminal, which synthesizes and secretes the contents of vesicles that contain the transmitter, acetylcholine (Ach), and (ii) the postsynaptic terminal, which binds transmitter for transduction of information. The arrival of the motor neuron action potential stimulates membrane-mediated processes to promote the migration of Ach vesicles to active zones located at the distal portion of the presynaptic terminal.
Exocytosis of a single vesicle liberates a quantum of active transmitter into the cleft, which subsequently bind to Ach receptors (Ach-R) on the postsynaptic membrane, facilitating end-plate depolarization and muscle contraction (Kandell and Schwartz, 1983).

The postsynaptic membrane is characterized by the presence of numerous folds, which bear crests occupied by Ach-R’s at an approximate density of 10,000 copies per square micron (Salpeter and Loring, 1985). Deep within the folds resides the membrane-bound enzyme acetylcholine esterase (Ach-E), which functions to hydrolyze Ach to choline, making the latter available for reuptake by the presynaptic terminal.

Biochemical analysis shows Ach-R’s as being composed of four polypeptide subunits, termed alpha, beta, gamma, and delta (Salpeter and Loring, 1985). The alpha subunit contains two binding sites which bind both agonists (including Ach) and antagonists (such as alpha-neurotoxins) with high affinity. The details of the binding characteristics of the alpha subunit have been greatly facilitated by the use of alpha-neurotoxin, such as the alpha-bungarotoxin extracted from snake venom. All subunits of Ach-R’s completely span the membrane and together have been hypothesized to form an ionic channel predominantly permeable to cations. The opening and closing of these channels is dependent upon Ach binding. Under stimulated conditions, acetylcholine bind to Ach-R’s, which promotes transient channel opening and depolarization of the motor end plate. These end plate potentials can be recorded at the NMJ by the use of KCL-filled microelectrodes. Under resting conditions, smaller depolarizations can be observed as a result of the occasional exocytotic discharge of the contents of a storage vesicle (Hess, et al, 1983). These small randomly occurring depolarizations are known as miniature end-plate potentials (mepp) and are usually of a consistent amplitude, which
supports the exocytotic mechanism of transmitter release. It has been estimated that about 30 vesicles of transmitter must be released into the cleft to produce an epp which is above the threshold (typically 15mv depolarization) for the initiation of an action potential in the skeletal muscle fibre (Kandell and Schwartz, 1983). Once the action potential has been initiated it will propagate along the surface of the sarcolemma and cross sectionally through the transverse tubules. Junctioned along the transverse tubules are membrane structures known as sarcoplasmic cisternae, which when activated by the arrival of an action potential liberate calcium that is important in promoting acto-myosin interaction and skeletal muscle contraction. (Best and Taylor, 1986).

The mechanisms of channel opening and closing have been difficult to elucidate because the Ach receptors demonstrate desensitization under activated conditions. Desensitization is a term that describes the observation that, the post-synaptic receptors lose their response to Ach after relatively prolonged exposure (Thesleff, 1959).

The anatomical and physiological properties of the NMJ described here stimulate speculation as to a number of areas within the NMJ that may be subject to failure. As such, both pre-synaptic and post-synaptic mechanisms have been proposed. Rather than investigate these mechanisms in a chronological order we will review the literature starting with studies dealing with the motor axon and work distal to the sarcolemma.
0.5.1 Animal Studies

The motor axon is unlikely to fail during activation since isolated nerve preparations can last through several hours of stimulation under normal conditions (Krnjevic and Miledi, 1958). However, Krnjevic and Miledi (1958) found that nerve axons innervating the mouse diaphragm were sensitive to temperature changes (both cooling and heating) and hypoxia, conditions that might prevail as the distal portions of the axon enters the muscle fasciulus. The portion of the motor axon that has shown potential to fail are the bifurcation points which divide the axon to innervate different muscle fibres. Evidence to support this hypothesis was first presented by Krnjevic and Miledi (1958, 1959) in experiments using rat phrenic nerve-diaphragm preparations stimulated via the nerve at frequencies between 10–50/s. Recordings of endplate potentials (e.p.p.) from two endplates of the same motor unit showed asynchronous failure of e.p.p.'s. They rejected a lack of Ach production based on unpublished data and hypothesized branch point failure. Hatt and Smith (1976) directly addressed this hypothesis by stimulating a crayfish opener nerve-muscle preparation. Two observations from this study convincingly supported branch point failure. Firstly, the nerve was stimulated at 30 Hz while compound action potentials were recorded from the axon distal to the point of stimulation. After a period of activation the compound action potential was shown to reduce in size, with the magnitude of the reduction correlating to the amplitude of a single nerve action potential. Secondly, when the recording electrodes were moved to an area very close to the nerve terminal, where excitatory nerve terminal potentials (ENTP's) were recorded, it was observed that 4 of 11 ENTP's of a common motor unit failed in response to the same stimulus. The conclusion was that action potentials failed to be propagated.
down certain axons subsequent to bifurcation. Recording from the pre-synaptic terminal allowed Hatt and Smith to eliminate failure of more distal processes, which Krnjevic and Miledi (1958) were unable to do recording from the endplate.

The ability of the nerve terminal to maintain adequate output of Ach has been a topic of discussion in many papers, however, the number of studies actually conducted to test the integrity of this system have been few. This has been primarily a result of the difficulty in measuring changes in Ach concentrations during repetitive activity. Krnjevic and Mitchell (1961) attempted to monitor Ach release from the rat diaphragm in response to phrenic nerve stimulation. Their results demonstrated that during high frequency stimulation Ach levels were depressed. However, their findings were based on bio assays that showed high degrees of variability ($\pm 20\%$ to $\pm 50\%$), as well, these assays relied on assumptions that may not be acceptable today. The Ach that was assayed was extracted from the bathing solution which likely did not contain Ach as its only active ingredient, since anticholinesterases and curare were used. Furthermore, the Ach extractant can only represent the Ach concentration liberated by a mass group of active terminals. To confidently be able to determine that the observed reduction in Ach was due to a reduction in the synthesis and release of Ach, information from single axon terminals must be evaluated. Brooks and Thies (1962) isolated single axon terminals of guinea pig serratus muscle using intracellular microelectrodes implanted into the endplate. Ach release per impulse was estimated by the analysis of the coefficient of variation of a series of e.p.p. amplitudes. Their results agreed with Krnjevic and Mitchell (1961) that Ach release was depressed in response to nerve stimulation, and the depression was of greater magnitude when the rate of stimulation was high. The same conclusions were reported by Elmqvist and Quastel (1965) in which e.p.p.'s
were recorded from human intercostal muscle in response to nerve stimulation. Calculation of Ach changes was similarly derived from the variance in e.p.p. amplitude. While this method of analysis demonstrated a reduction in the amplitude of the e.p.p.'s for both the above studies, it does not necessarily reflect a problem with synthesis and release of Ach. Other mechanisms that involve the fate of Ach in the synaptic cleft and changes in the properties of the postsynaptic membrane must be considered.

Once Ach is released into the synaptic cleft it has essentially three fates; (i) it can diffuse into the extracellular medium, (ii) it can bind to the postsynaptic membrane for the production of e.p.p.'s, or (iii) it can be broken down into acetyl CoA and choline by membrane bound cholinesterases. Each of these processes are regulated by rate constants as reported by Kordas (1972). By first using a computer model Kordas was able to alter a simulated endplate current through manipulation of the rate constants. He then applied this principle to a sciatic nerve-sartorius muscle of the frog by inhibiting cholinesterase with prostigmine, allowing a greater amount of Ach to reside in the cleft. This procedure resulted in the prolongation of the falling phase of endplate currents, and since e.p.p.'s are graded potentials the probability of temporal summation is enhanced. Translated this suggests that Ach may be limiting and by increasing the amount of available Ach the probability of producing a muscle action potential is greater. These data, however, may not be directly relevant to fatigue since the study was not designed to investigate fatigue mechanisms, and the stimulation protocol was not present in the methods. Nonetheless it provides some insight into the affects anticholinesterases have on transmission, which will be discussed later.
It was mentioned earlier that even if an Ach molecule remains bound to a post-synaptic receptor the ion channel will not remain open. This was termed desensitization. If this process exists then it is possible that the reduction of the e.p.p.'s observed by Krnjevic and Miledi (1958, 59), Brooks and Thies (1961) and Elmqvist and Quastel (1965) may have resulted from desensitization of the motor endplate. Thesleff (1959) investigated this speculation with stimulation of the isolated phrenic-diaphragm preparation of the rat. The preparation iontophoretically received applications of known doses of Ach. After a period of high frequency stimulation (30-60 Hz) the response of the preparation to the test dose of Ach was greatly reduced compared to pre-stimulation trials, suggesting that the diaphragm became less sensitive to Ach. Further work relating desensitization to transmission failure has not, to my knowledge, been published.

In Krnjevic and Miledi's 1958 study on the mouse diaphragm the e.p.p.'s were shown to decrease in amplitude, as well as drop out completely in response to high frequency stimulation of the phrenic nerve. Recording electrodes were also implanted into the sarcolemma of the muscle fibres, where it was observed that the action potential threshold increased during stimulation. The combination of a reduced e.p.p. and an increased threshold make it increasingly difficult to initiate a muscle action potential. Thus the ability of the sarcolemma to initiate and maintain the muscle action potential has been questioned. It is well known that the certain characteristics of the muscle action potential change with stimulation of isolated preparations, as well as in humans during both voluntary and non-voluntary contraction. During repetitive stimulation of a single motor unit, the action potential becomes distorted in shape, having a smaller amplitude and a broader time base (Sandercock, et al 1985, Clamann and Robinson, 1985, Hatt and Smith, 1976, Juel, 1988). This may be due to a reduction in the conduction
velocity of the action potential (Bigland-Ritchie, 1981, Juel, 1988, and Milner-brown and Miller, 1986), a downward shift in the power spectrum (Viitasalo and Komi, 1977), and the environmental conditions of the muscle fibres (Krnjevic, 1958, and Luttgau, 1965). The latter was investigated by Luttgau (1965) in which action potentials were recorded from the semitendinosus and the iliofibularis of English frogs. Again, high frequency stimulation (as opposed to low frequency stimulation) induced changes in the wave form of the individual action potentials, which was followed by the complete drop-out of some impulses entirely. When glycolysis and oxidative metabolism were blocked using sodium cyanide and iodoacetate limiting the production of metabolic by-products, the sarcolemma showed little action potential fatigue and responded more like a nerve (Luttgau, 1965). This study suggested that fatigue of the action potential is a consequence of metabolic reactions associated with muscle contraction. If the sarcolemma fails to produce action potentials the force generating capacity of the muscle is halted.

It is clear from the above discussion that both pre-synaptic and post-synaptic events fail during high frequency repetitive stimulation. The questions that remain are: Do the same events fail during voluntary activity in animals?, and is transmission failure occurring in humans during either voluntary exercise or through stimulation?. Experiments to elucidate the former have not been challenged, probably because experimentation of this nature would be extremely difficult in animals. Studies to investigate the second question have been attempted several times since the early 1950's with limited success. The major problem that has inhibited successful study of neuromuscular transmission in humans, has been the difficulty in observing and quantifying any change that occurs within the NMJ during muscular activity. Introduced earlier
was electromyography, which has provided the only tool for the study of neuromuscular transmission. This technique, whether it’s being recorded from the surface of the muscle or from indwelling electrodes, yields very limited information about activity at the NMJ, and in no way can mechanisms be derived from its analysis. Nonetheless electromyography stands as the only method available for noninvasive study of neuromuscular transmission.

0.5.2 Human Studies

One of the first to employ electromyography for the study of muscle fatigue was Merton in 1954. The study involved human subjects who had their wrists supported in a special apparatus that isolated the adductor pollicis muscle of the hand. Maximal static effort of the adductor pollicis was sustained for about two minutes, while single maximal shocks were applied to the ulnar nerve throughout the course of fatigue. Merton observed that as force fell the superimposed nerve shocks were unable to restore any of the lost force. Furthermore the EMG records (M-wave) did not reveal any evidence of electrical failure. These data led to the conclusion that the central drive and the neuromuscular junction maintain normal function, and that the force generating capacity during fatigue is limited by failure of events peripheral to the sarcolemma. Merton’s conclusions were challenged several years later by Stevens and Taylor (1972) in a similar experiment using the first dorsal interosseous muscle of the hand. Their experiments resolved two phases of fatigue; the first, lasting about one minute, where the tension and EMG fell in parallel; and a second phase subsequent to this, where the force fell much faster than EMG.
The M-wave amplitude was shown to be reduced by 65%, with the bulk of this reduction occurring during the first phase of fatigue. These findings were interpreted to suggest that during the first phase of fatigue, failure of neuromuscular transmission was responsible for the force loss, while contractile mechanisms were responsible during the second phase. From this they postulated that since high threshold FF units generate the greatest peak tensions and fatigue first, that these units are more likely to demonstrate transmission failure than the S-type units. This view has been shared by others (Pagala, 1986, Clamann, 1985, Enoka, 1985, Kugelberg and Lindegren, 1979, and Grimby, et al, 1981) who have observed transmission failure in stimulation experiments on animal FF-units. Furthermore, these units respond to high frequency activation, and it has already been disclosed from invitro studies discussed earlier, that a prerequisite for transmission failure are high rates of stimulation (Krnjevic, 1959). Whether FF-type units in humans demonstrate different patterns of electrical failure than S-type units cannot be resolved by previously completed experiments, since the data form these studies have been extracted from muscles of the hand, which are predominantly of S-type composition.

The conflicting evidence presented by Merton (1954) and Stevens and Taylor (1972) was addressed by Bigland-Ritchie (1982) in an experiment in which the protocols of both authors were repeated on the adductor pollicis and the first dorsal interosseous muscles. The results of this experiment agreed with the findings of Merton (1954) demonstrating that for both muscles there was a 30-50% reduction in tension that was not accompanied by any decline in the M-wave. There was, however, a disagreement between Bigland-Ritchie's data and those of Stevens and Taylor, which appears to stem from the difference in methods used to analyze changes in the M-wave. Bigland-Ritchie used four
methods to monitor the M-wave: (i) the peak to peak amplitude; (ii) the total area above and below the isoelectric line; (iii) the area of the first half of the wave only; and, (iv) the area over a specific time interval of the M-wave, which was the method used by Stevens and Taylor (1972). The peak to peak amplitude was observed to decline with fatigue, however, it was discovered by Bigland-Ritchie that if the stimulating electrodes were moved slightly there was no reduction observed, suggesting that the decline in amplitude probably resulted from electrode shifts over the course of exercise. This discrepancy could have been avoided if Bigland-Ritchie used higher stimulation voltages, since it is questionable whether supramaximal levels were reached. They stimulated the ulnar nerve up to 75 volts, while Stevens and Taylor used much higher voltages of up to 400 volts. At such high voltages small shifts in the stimulating electrode would not likely affect M-wave amplitude. Bigland-Ritchie also only stimulated the preparation with single shocks, while Stevens and Taylor applied two pulses 20 ms apart. Stevens and Taylor used the second pulse to analyze the M-wave, thus their results may not be directly comparable to Bigland-Ritchie's, since the first pulse effects the output of the second pulse (Burke, 1976). The other methods Bigland-Ritchie used to analyze the M-wave showed no changes in the whole or half wave areas, but when the method of Stevens and Taylor was used a similar reduction in the M-wave was observed. Bigland-Ritchie concluded, however, that the method of Stevens and Taylor neglects to account for the slowing of conduction velocity of the action potential that is known to occur during repetitive activation (Milner-Brown and Miller, 1986). The action potential becomes flatter, and thus the area between the two defined points in time will become smaller. This reduction, therefore, can only be attributed to changes in the appearance of the action potential and whether or not these changes reflect failure of transmission cannot be answered with those data. Intramuscular
recordings from single motor units of the adductor pollicis (Bigland-Ritchie, 1982), however, showed more reliably that the spike area was maintained during 60 seconds of contraction. Therefore the study presented by Bigland-Ritchie (1982) supported the findings of Merton (1954), but did not adequately reject the work of Stevens and Taylor (1972). Since Bigland-Ritchie's 1982 study others have investigated the same problem. Kukulkia, Russell, and Moore (1986) found that the M-wave area was maintained for up to three minutes of maximal exercise of the human soleus, despite a 30% reduction in tension. In 1988, however, Bellemare and Garzaniti conducted a well controlled study that involved maximal exercise of the adductor pollicis. This study demonstrated that the decrease in tension and EMG that naturally occurs during fatigue was also accompanied by a parallel decrease in the M-wave amplitude.

The question of whether failure of neuromuscular transmission occurs in fatigueuing exercise still clearly remains unresolved. Further research is warranted by the fact that failure of both pre-synaptic and postsynaptic events have been well correlated to force loss in isolated nerve-muscle preparations in animals. More importantly, however, it has been shown that certain disorders that affect neuromuscular transmission led to symptoms similar to exercise-induced fatigue. Historically, the analysis and understanding of pathological conditions have often led to a better understanding of the normal functioning of physiological systems. Of particular interest to this study is the disease myasthenia gravis (MG). This disease is an autoimmune disorder of the NMJ in which antibodies are directed against Ach-R's of skeletal muscle resulting in symptoms of weakness and easy fatiguability. In some cases the disease only affects the extraocular muscles, in which case it is termed 'ocular MG", but most individuals have a wide-spread set of symptoms, and are said to have "generalized MG" (Seybold, 1983).
Originally patients who demonstrated the classic symptoms of myasthenia gravis were diagnosed with the anti-cholinesterase, neostigmine, a drug that produced several uncomfortable side effects lasting up to several hours (Flake, 1973). In 1952, however, Kermit and Kaplin introduced a new rapid diagnostic test for MG by intravenously injecting edrophonium chloride (tensilon). Within one minute of injection, this drug provided transient alleviation (5–10 min.) of fatigue in people with MG. This observation provides an alternative approach to the investigation of neuromuscular transmission in normal humans. The studies discussed earlier have relied on the analysis of surface EMG to indirectly monitor transmission, which has proven suspect to many uncertainties. The use of edrophonium chloride provides a more direct method of investigation of transmission by allowing the researcher to alter Ach levels in the cleft of the NMJ. Furthermore the short half life and easy application of edrophonium renders this drug suitable for experiment on exercising humans.

In skeletal muscle, the manifestations of MG are similar to those of fatigue induced by exercise. This disorder of the NMJ is successfully treated with anti-cholinesterases, therefore it can be predicted that if fatigue is delayed by edrophonium chloride, NMJ failure is a likely cause of exercise-induced fatigue. Two experiments were designed to investigate this prediction. The first experiment tested the effects of edrophonium chloride on force loss and electrical failure that accompany fatigue from maximal voluntary effort (both dynamic and static exercise) of the knee extensors. The second experiment followed essentially the same exercise protocol, however, prior to the actual drug tests the subjects were pre-fatigued such that they were unable to produce their absolute peak tensions. This is a symptom that is experienced by the myasthenic patient, thus experiment two was designed to test the effects of edrophonium
chloride under conditions that somewhat simulate MG.

Edrophonium acts as an anticholinesterase, that when injected will promote an acute enhancement of ACh in the cleft of the NMJ (Seybold, 1983, and Katz, N., 1967), and hence increase the probability of ACh-R binding. This drug is a synthetic quaternary ammonium ion (shown in figure 4) that reversibly attaches to the anionic site of the cholinesterase molecule. Cholinesterases are not only found at the NMJ, but are widespread throughout the abdominal visera and organ systems associated with ACh-R's, termed muscarinic receptors. These receptors function to mediate signals from the parasympathetic nervous system and differ in some of the binding characteristics associated with the skeletal muscle nicotinic receptors (Kandell and Schwartz, 1983). As with the nicotinic receptors, edrophonium chloride enhances muscarinic receptor activity, however with activation of the latter a variety of autonomic physiological responses follow. These may include; nausea, vomiting, diarrhea, miosis, excessive salivation.

Figure 4. Chemical structure of edrophonium chloride
sweating, increased bronchial secretions, abdominal cramps, hypotension, and bradycardia. These side effects may cause discomfort in some subjects, as well as reduce the reliability of the double blind nature of this study. Under clinical diagnostic conditions patient discomfort is usually minimal and the synergistic injection of a muscarinic antidote is seldom required (Roche, 1986, Ojer, 1986, and Cronnelly, 1982). However, for the purposes of this experiment, the muscarinic blocker atropine will be injected in small doses along with edrophonium. Atropine will block all parasympathomimetic side effects induced by edrophonium, and although it will provide a good control, it is also accompanied by side effects. The adverse effects of atropine are of an anti-parasympathetic nature and are dose related: with 0.5 mg, slight cardiac slowing, some dryness of the mouth, and inhibition of sweating may be noticed; at 1.0 mg, dryness of the mouth, thirst, increased heart rate and mild pupillary dialation are common; and above 2 mg, the above responses are potentiated and may also be accompanied by difficulty in micturition, flushed skin, blurred vision, reduced intestinal peristalsis, headaches, and restlessness (Weiner, 1985). With the use of atropine and by knowing that edrophonium does not cross the blood brain barrier, the assumption of this experiment will be that any drug effect of edrophonium will be at nicotinic sites.
PART B

HYPOTHESES
0.6 Experiment one

The purpose of experiment one was to investigate the effects of edrophonium chloride on force loss and electrical failure during fatigueing exercise of the knee extensors. Four force variables and one EMG variable were of statistical interest to this study. With respect to force, the following predictions were forwarded:

1. The time to onset of fatigue (TTO), defined as the time to 90% MVC, will be prolonged with the administration of edrophonium chloride.
2. The time to fatigue (TTF), defined as the time to 55% MVC for dynamic exercise and 60% MVC for static exercise, will also be prolonged with the administration of edrophonium chloride.
3. For the final force analysis a common time between the drug and control was chosen (the actual time being the maximal latency common to both conditions), and the percent MVC at that specific time will be compared (TS%MVC). It is hypothesized that the TS%MVC would be higher as a result of administration of edrophonium chloride.

To produce force, an EMG signal must be present; however, to observe an EMG signal does not require the production of force. From this we postulated two outcomes for the EMG:

1. Edrophonium chloride will increase the overall integrated surface EMG values, and if 1 and/or 2 above are true, then transmission failure is a likely mechanism in fatigue.
2. If 1 and 2 above are rejected and edrophonium chloride increases EMG activity then transmission failure is unlikely and fatigue is likely to involve
mechanisms distal to the sarcolemma.

0.7 Experiment two

Experiment two was designed to simulate one symptom of the neuromuscular disorder myasthenia gravis—the inability to produce absolute maximal tension through maximal effort. Statistical analysis was completed on the same variables as in experiment one, thus the hypotheses of experiment one also apply to experiment two. The primary question to be answered in experiment two is, whether or not edrophonium chloride restores the ability to achieve maximal tension in pre-fatigued muscle. We hypothesize that edrophonium will restore peak tensions to values that are significantly higher than the controls and are comparable to the peak tensions of experiment one.
PART C

EXPERIMENT ONE
0.8 Methods

12 male volunteer subjects were paid to participate in this study subsequent to medical approval as determined by D. Hedges, M.D. Medical approval consisted of a questionnaire, an examination and a test injection for sensitivity to edrophonium chloride. Informed consent was obtained, after which the subjects where trained to perform both static and dynamic exercise with the quadriceps muscle using a Cybex knee extension/flexion machine. For the experimental trials, edrophonium chloride and atropine sulphate, or atropine sulphate alone (control) was administered intravenously in a randomized, double-blind fashion to investigate the effects of the former drug on force output and IEMG.

0.8.1 Apparatus

All exercise conditions were carried out on a Cybex II Isokinetic Dyanometer (Lumex Inc., Cybex Division, New York). This machine was chosen because of its versatility in providing measures of muscular output at pre-selected controlled velocities ranging from zero degrees/s (static contraction) to over 200 degrees/s (dynamic contraction). Furthermore, for the purposes of this study the Cybex is ideal for testing an individual's repeated ability to sustain high levels of muscular performance. It was recognized that the reliability of the Cybex has been questioned (Johnson and and Siegel, 1978; Winter, D., et al., 1981; and Murray and Harrison, 1986) since several errors may be introduced when assessment of absolute muscle function is required. The major errors of concern are in; calibration, signal filterization, and inertial and gravitational
corrections. These errors present a problem if calculation of the "real" muscle forces, power and work are to be analyzed. However, in the present study, fatigue was monitored simply as a function of the relative change in force measured over time. The force recording represents the total force applied by the subject against the lever arm, and since the subjects performed repeated measures where the mechanical errors are the same for all conditions, the Cybex provided a reliable assessment of the drug effect.

The force output was converted from mechanical torque applied at the lever arm to an electrical signal which was recorded on a Cybex II Dual-Channel Pen recorder. Calibration procedures were carried out at gain settings of 180 and 360 ft-lbs/full scale deflection (paper speed at 5 mm/second) as described in the Cybex manual.

With the padded Cybex chair fully reclined and the subjects in the supine position, the right leg was oriented for knee extension and flexion. The natural axis of the knee joint and the mechanical axis of the Cybex lever arm was aligned such that the lateral condyle of the femur corresponded with the center of the lever arm axis. A constant lever arm distance was provided by positioning a padded velcro strap exactly 34 cm from the axis of rotation to the center of the pad support bar. Additional straps were secured around the thigh, waist and shoulder to provide support for those regions and to ensure optimal isolation of the quadriceps.

Both edrophonium chloride and atropine sulphate affect cardiac function, thus it was necessary to monitor heart rate for the duration of all drug trials. The electrocardiogram was set up in the traditional triaxial reference system, with a ground electrode positioned over the inferior surface of the right scapula, a positive lead placed over the lateral surface of the left lower chest and a
negative lead placed over the lateral surface of the right chest. In addition to monitoring heart rate D. Hedges, M.D., was always present in case of a medical emergency.

Muscle electrical activity was monitored over two areas of the quadriceps, the vastus lateralis and the rectus femoris. Two sites were chosen primarily to provide a greater index of the overall electrical activity of the fatiguing quadriceps muscle. The electrical activity was recorded with Silver-silver chloride electrodes placed 5 cm apart over the approximate motor areas of the vastus lateralis and rectus femoris. To achieve minimal resistance, the skin was shaved, scrubbed with isopropanol, and received an application of conduction jelly. After the first exercise trial was completed, the skin was marked with water resistant ink so that during successive trials, the electrodes were positioned in the exact same place. Excess 60 Hz line noise was eliminated with a large ground electrode, constructed of wire wrapped with gauze, soaked in a saline solution and secured to the exercising leg just above the calf.

The EMG signal from both muscle groups were amplified independently through two differential microelectrode preamplifiers (model P15, Grass Medical Instruments, Quincy, Mass., U.S.A.) These amplifiers are equipped with a high input impedance and low noise (typically 5 microvolts over a bandwidth of 0.1 to 300 Hz), a maximal gain of 1000, and a frequency response of 0.1 - 50 KHz. For these experiments amplification was set at 100 times, and the frequency cutoffs at 30 and 3000 Hz. Prior to each test three calibration signals of 1 mV were collected from the preamplifiers.

The amplified EMG was then split, with one BNC lead attached to a dual beam oscilloscope (Tektronic t921) for verification of a clean EMG signal, while a second lead was relayed to a portable instrumentation FM tape recorder (model...
PI-6200) for storage of a hard copy of the analog signal. The specifications of this recorder were adequate for these experiments having an input impedance of 50k ohms or greater, a frequency response of 5Hz-1000Hz at a tape speed of 3.75 ips, and a signal to noise ratio of 38 db (Precision instrument manual 3170 Porter drive, Palo Alto, California).

After all data collection was complete the information saved on the tapes were then AC filtered and rectified (removing DC bias) and subsequently digitized. The digitization process was completed through an IBM A/D conversion unit, which required specific program modifications (Rob Taylor, 1988) of IBM labpac (see appendix for program details). The program was designed to sample the analogue signal at a frequency of 500Hz which, avoids aliasing of EMG signals up to 250Hz. For data extracted from the dynamic trials, the computer was programed to save information only during the extension phase. This was accomplished by setting a trigger level that was sensitive to changes in EMG amplitude above basal levels. We were confident that the digitization process was accurately extracting only the extension phase of EMG since we simultaneously received visual feedback of the EMG from an oscilloscope, which was synchronized with the collection cues visible on the computer screen. Audio feedback from the tape recorded could also be used from varification of proper computer synchronization. Reliability tests were also performed, in which the data were sampled four times over the same section of tape. The results of these trials can be found in Figure 6 in the appendix. For the static trials the computer saved one second samples every two seconds. The details of the program design for this procedure can also be found in the appendix. After the data were digitized the computer integrated each extension phase of the dynamic trials, and the one second segments of the static trials.
Figure 5. Schematic representation of experimental set-up.
The majority of studies that have been conducted to investigate fatigue have presented results that are specific for isometric contraction only. From these studies generalized statements regarding muscle fatigue must be taken with caution since under normal physiological conditions during movement, static muscular contractions are rarely observed. The obvious concern between static and dynamic exercise that would effect the course of fatigue is the lack of blood flow to the muscle that accompanies isometric contractions. This lack of perfusion will likely lead to a greater build-up of contractile by-products, as well as increase the oxygen debt, compared to dynamic contraction where blood flow is not limiting. Since both the lack of oxygen and the build-up of metabolic wastes are known to have detrimental effects on muscle function (Stevens and Taylor, 1972 and Belcastro, 1985), the course of fatigue is likely to differ significantly between static and dynamic contractions. Furthermore, the patterns of motor unit firing differs, with the dynamic contraction showing a cyclic pattern, while a constant firing pattern is required to maintain tetany during static contractions. From these observations we feel that if generalized conclusions of muscle fatigue are to be made both dynamic and static conditions should be considered. Consequently, during our experiments both dynamic and static exercise protocols will be followed.

An endurance protocol (Cybex testing protocol, Lumex Inc., 1975) has been designed for the Cybex to quantify muscular fatigue during repetitive, maximal contractions. For the dynamic condition an angular velocity of 180 degrees/s was selected because it provided maximal dynamic freedom without challenging the accuracy of the Cybex at its upper most limit of 200 degrees/s. Furthermore, in
a report by Johnson and Siegel (1978) on the reliability of isokinetic movement of the knee extensors at 180 degrees/s, correlation coefficients ranging from .93-.99 were demonstrated between trials over a 5 day period. Also, Barnes (1981) compared isokinetic fatigue curves at different angular velocities, and demonstrated that at high angular velocities (between 150-300 degrees/s) subjects fatigue more rapidly.

The angular displacement of the leg during the dynamic exercise ranged from a 90 degree joint angle to 0 degrees when the leg is fully extended. The full range of motion was ensured by having the subject make contact with a board that was adjusted in front of the subject at a height corresponding to the extended leg position. This also helped to maintain the subject’s motivation for maximal contraction, as the subject was encouraged to kick the board with maximal effort.

Each subject performed three practice trials to become accustomed to the feel of the accommodating resistance provided by the Cybex. These trials lasted 45s with two minute rest periods between trials. During the experimental trials, which were conducted on separate days, the subjects performed one set of maximal reciprocal contractions until his force output had fallen to 55% of the starting value. This level of fatigue was chosen because it was a value reached within a reasonable time period (30-60s) as well it was a level that minimized subject discomfort that was associated at higher fatigued states.

For the isometric condition the subject was positioned on the Cybex machine with the joint angle about the exercising knee at 120 degrees and the angular velocity of the speed selector unit set to zero. At this joint angle Murray et al (1977) demonstrated that the highest isometric torques could be achieved, as compared to other joint angle over a 90 degree range.
The subjects also performed three static practice trials prior to the drug testing. These were conducted on the same day as the practice sessions for the dynamic condition, and consisted of three MVC's also lasting 45s with two minute rest periods between trials. Under experimental conditions the subject performed a maximal voluntary static contraction until the force output had decreased by 40%. Preliminary testing revealed that the fatigue was slower during static exercise, as well the subjects reported greater discomfort. For these reasons the fatigue index differed slightly between dynamic and static exercise.

On the day of the experiments, testing commenced after the electrocardiogram and electromyogram electrodes were in place and normal recordings were observed. All drugs were administered by intravenous injection in a randomized, double-blind fashion and maximal exercise was performed exactly 2 minutes after the completion of the injection. Preliminary experiments demonstrated that the subjects were never able to produce their highest peak tensions during the first 3-5 seconds of static exercise or until about the third dynamic contraction. This was overcome by having the subject perform three "build-up" contractions prior to starting the timer. For the "build-up" contractions the subjects qualitatively contracted the first at about 25% MVC, the second at 50% MVC, and the third at about 75% MVC. Under these conditions the fourth contraction, which was designated time zero, was, on average, the maximal peak tension. The subjects received vocal encouragement from the experimenter to perform maximally.
0.8.3 Drug conditions

1. Control........0.3 mg Atropine sulphate + normal saline
2. Drug..........10 mg Edrophonium chloride + 0.3 mg Atropine sulphate

Both drugs act rapidly (within 1 minute) and have short half-lives. Peak edrophonium chloride activity occurs between 0.8 and 2.0 minutes (Flake, 1973). In order to ensure peak drug activity during the experiments, exercise trials did not proceed until two minutes had elapsed after injection. The effects of this drug usually do not last longer than 10 minutes. Atropine's peak activity is also rapid (1-2 min), and any noticeable effects of atropine (which was limited to dryness of the mouth at the above dosage) dissipated 1-2 hours after injection. However, as a precaution, subjects were requested to remain in the lab for 30 minutes after the conclusion of the exercise.

The drugs were administered at doses far below toxic levels, and edrophonium sensitive subjects were screened with a test injection during the medical examination. Clinically edrophonium is intravenously injected in 10 mg doses, and for precautionary reasons 1 mg is injected first, during which 30 seconds must elapse with no adverse side effects before the rest is injected over the next 30 seconds. This procedure was not required during the experiments since the subjects were pre-tested for adverse reactions. Don Hedges, M.D. administered all drugs and medications, and provided constant observation of the subjects during all drug trials.

The subjects were requested to complete four individual days of testing, which consisted of 2 dynamic (control and drug conditions) and 2 static trials (control and drug). They were also asked, that during period of testing to refrain
from any heavy leg training, as well as to refrain from caffeine or any other stimulants for three hours before the experiments.

0.9 Data Preparation, Analysis And Statistics

The force data for experiment one and two were prepared for statistical analysis in the following way: A ruler with a millimeter scale was used to measure the deflection (top of baseline to top of deflection) of the force tracing to the nearest 0.5mm, as shown in Figure 7 in the Appendix. The obtained value was converted to S.I. units using the following formula: (measured value in mm) \( \times \) (gain factor in ft-lbs)/ 50mm \( \times \) (0.3M/ft) \( \times \) (0.45Kg/lbs) \( \times \) 9.81 M/s\(^2\) = Force in newton-meters. To normalize the data, all corrected values were divided into the peak value and multiplied by 100. The TTO, TTF, TC and TS%MVC were then calculated and the drug vs. control conditions were analyzed using a standard Student's T-test for repeated measures.

The raw EMG signal was first saved on FM tape and later digitized, as described earlier. The digitized EMG signal was corrected for the 100 times amplification and respective calibration value. These values were also normalized as described above. A single value representing the total integrated EMG for TTF was calculated using the following formula: \( I_{EMG} = [(EMG_{t1} + EMG_{t2})/2 \times (t2-t1)] + [(EMG_{t2} + EMG_{t3})/2 \times (t3-t2)] \)...... where t is the time at which the respective EMG values were recorded. These \( I_{EMG} \) values for the drug vs. control conditions were also compared using a one-way ANOVA for repeated measures, which compared both drug conditions, as well as muscles groups.
0.10 Results

The pharmacological action of edrophonium chloride results in the enhancement of acetylcholine levels in the synaptic cleft at both nicotinic and muscarinic junctions. To secure the double-blind nature of the experiments, 0.03 mg of atropine was administered with both the control and drug injections. Indirect conformation that these drugs were active in the system was extracted from the heart rate data of experiment one. Figure 8 illustrates the change in heart rate during the two minute absorption subsequent to the injections. The lower curve represents the edrophonium chloride plus atropine trials (n=24), and Figure 8. Heart rate vs. time from data collected in Experiment 1 during the two minute drug absorption period prior to exercise.
clearly shows that the edrophonium chloride induced a significant drop in heart rate. The average resting heart rate was 58.0 ± 11.1 beats/minute, but after 48 seconds the rate fell to 55.9 ± 10.7 beats/minute where it became significantly different from the atropine plus normal saline trials. After 84 seconds the heart rate was lowest at 49.7 ± 11.3 beats/minute, but then began to rise, probably in anticipation of exercise or perhaps through a built in safety mechanism known as vagal escape. If the heart rate drops to low as a result of vagal stimulation the sympathetic nervous system shows increased activity which over rides the effect of the vagus. The atropine in the control injections promoted a significant rise in heart rate from a resting level of 59.5 ± 9.7 beats/minute to 74.4 ± 14.0 beats/minute after two minutes.

Comparisons between the static and dynamic exercise were not statistically acceptable due to the different protocols, however, there were certain features of the force data that distinguished the two conditions. Firstly, it was reported by all but one subject that the static exercise was far more uncomfortable than dynamic exercise, and that they felt more fatigued after static exercise than dynamic. Secondly, the peak tension for static contractions was on the average twice that of the dynamic contractions (255.0 ± 59.6 newton-meters and 142.0 ± 16.7 newton-meters respectively). And third, the rate of fatigue taken over the linear portion of the curve from figures 9a and 9b demonstrate that the subjects fatigued faster with dynamic exercise compared to static (66% MVC/minute and 50% MVC/minute, respectively).

The force variables, PT (peak tension), TTO (time to onset of fatigue), TTF (time to fatigue), TC (time course), and TS%MVC (the time specific level of force) are shown in Table 1 and illustrated in figures 10a to 10b. Statistical analysis revealed that edrophonium chloride had no effect on any of the force
Figure 9. A. Pooled results of force vs. time data for dynamic knee extension. B. Force vs time for static exercise. (Error bars not included)
variables described. These data do not support our hypotheses that the drug will delay fatigue, and instead supports the work of Bigland-Ritchie (1978, 1982), and others that transmission mechanisms are intact during MVC of human skeletal muscle.

Electrical fatigue during the maximal knee extension was accessed through EMG recorded over the belly of the vastus lateralis and the rectus femoris. The raw signal varied in amplitude between 5 mV and 12 mV with the larger values generally observed over the vastus lateralis, which was probably due to the

<table>
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<td>Control</td>
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<td>TTO(s)</td>
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<td>TC(s)</td>
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<td>TS%MVC</td>
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<td>49.9 ± 7.3</td>
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<td>60.2 ± 6.2</td>
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<td>PT(N-M)</td>
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<td>141.9 ± 16.6</td>
<td>262.5 ± 51.0</td>
<td>255.0 ± 59.6</td>
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<tr>
<td>IEMG RF</td>
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<td>3741 ± 538.8</td>
<td>2189 ± 521.6</td>
<td>2276 ± 394.7</td>
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<tr>
<td>IEMG VL</td>
<td>3673 ± 629.0</td>
<td>3535 ± 406.6</td>
<td>2291 ± 371.4</td>
<td>2344 ± 400.8</td>
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**Table 1.** Values for Time to onset of fatigue (TTO), time to fatigue (TTF), time course of fatigue (TC), the fatigue level at specific time (TS%MVC), the peak tension, and total IEMG (arbitrary units)(n=12, error=SD)
recording system (less fat resides over the vastus lateralis than the rectus femoris) rather than differences in the physiology of the muscle groups. The raw signal was integrated twice as described earlier. The first integration produced a plot of the EMG patterns (represented as percentage of the maximum value) over time and are shown in Figure 11. There was no subjective evidence from the IEMG curves that consistently distinguished the EMG of static exercise from the dynamic exercise. The second integration process produced a single value representative of the overall electrical activity of the particular muscle group. These values are presented in Table 1. There were statistically no differences observed between the vastus lateralis and the rectus femoris. Analysis of the effects of the edrophonium chloride on electrical fatigue were consistent with the force records demonstrating no significant differences between the control and drug condition. This rejects the hypothesis that the electrical failure that accompanies force loss is due to transmission failure across the NMJ.
Figure 10. A. Time to onset of fatigue (TTO). B. Time course of fatigue (TC). C. Time to fatigue (TTF). D. Peak tension. E. Time specific %MVC (T%MVC). (N=12, error=SD)
Figure 11. Pooled IEMG vs. time data. A. Dynamic control condition. B. Dynamic edrophonium. C. Static control. D. Static Edrophonium. (Error calculation see table one)
PART D

EXPERIMENT TWO
0.11 Methods

All of the procedures of Experiment 2 are identical to those of Experiment 1 with the exception of the following changes:

1. Prior to the drug injections the subjects were pre-fatigued with 40 maximal dynamic contractions. This procedure was conducted to create a situation similar to those experienced by the myasthenic patients; the inability to produce absolute maximal tension due to neuromuscular block.

2. At the end of the 40 contractions either the drug or the control was injectioned. After one minute had elapsed the subjects were requested to produce maximal dynamic or static contraction with no prior "build-up" contractions. We anticipated that since the subjects were already warmed-up with the pre-fatiguing protocol that the first contraction of the actual test would produce the peak tension. A one minute absorption period was used instead of two minutes, to reduce the recover time from fatigue. It was likely that peak drug activity was reached during this time since the subject had just completed 40 maximal contractions in which heart rate and blood flow were greatly increased.

0.12 Results

Experiment 2 differed from experiment one with the addition of 40 maximal dynamic contraction prior to drug administration and exercise. The pooled data for the pre-trials (Figure 12) illustrate the reproducibility of the fatigue curves.
over four separate days, and show that the forty contractions produced approximately a 60% reduction in force. The objective of the prefatiguing procedure was to create a situation where the subjects, with maximal effort, were unable to produce maximal force due to fatigue. This criterion was met as indicated by the reduction in the average peak tensions obtained in Experiment 2 compared to Experiment 1. The peak tension for dynamic exercise was reduced by approximately 25%, while for the static exercise the peak tension was reduced by 15%. The presence of edrophonium chloride did not restore the force loss since there were no significant differences in peak tension between the control and drugs condition. This was also the case for TTO, TTF, TC, TS%MVC as shown in Table 2 and figures 14 to 15, and for the IEMG shown in Table 2. Thus the findings of Experiment 2 support those of Experiment 1 that transmission failure does not limit force production during maximal fatiguing exercise of the knee extensors.
Figure 12. Pooled force vs. time data for the four dynamic pre-trials performed on separate days prior to either dynamic or static exercise.
Figure 13. A. Pooled force vs. time data for dynamic knee extension. B. Force vs. time for static exercise.
Figure 14. A. Time to onset of fatigue (TTO). B. Time course of fatigue (TC). C. Time to fatigue (TTF). D. Peak tension. E. Time specific %MVC (TS %MVC). (n=12, error=SD)
Figure 15. Pooled IEMG vs. time data. A. Dynamic control condition. B. Dynamic edrophonium. C. Static control. D. Static Edrophonium. (For error data see table 2)
Table 2. Values for Time to onset of fatigue (TTO), time to fatigue (TTF), time course of fatigue (TC), the fatigue level at specific time (TS% MVC), the peak tension, and total IEMG (arbitrary units). (n=12, error=SD)
PART E

GENERAL DISCUSSION
The present experiments were conducted to examine the speculation that neuromuscular transmission (NMT) failure accounts for some of the force loss during fatiguing exercise in humans. It has been proposed since the early 1950’s that transmission of the motor unit action potential is impaired under certain stimulation protocols, in animal nerve–muscle preparations. In humans, it can be confidently demonstrated that faulty transmission leads to muscle fatigue and weakness in patients suffering from myasthenia gravis. However, scientists who have investigated transmission failure in normal individuals have presented conflicting results. The disagreements have largely been due to the lack of technical flexibility available for the study of the intact neuromuscular system. In fact, electromyography (EMG) has provided the only quantitative tool where information about NMT can be extracted. As such, differences in methodology and data interpretation of EMG signals from the same or similar studies have yielded contradictory results. EMG data from Merton, 1954 (adductor pollicis), Bigland-Ritchie, 1982 (adductor pollicis and first dorsal interosseous), and Kukulka, 1985 (soleus) were interpreted to show no evidence of transmission failure, while Stevens and Taylor, 1972 (first dorsal–interosseous), and Bellemaire, 1988 (adductor pollicis), using very similar protocols to the above, both concluded that transmission failure influenced the course of fatigue.

Our approach to accessing transmission at the NMJ during fatigue was to attempt to enhance transmission using the cholinesterase blocker, edrophonium chloride. In two separate experiments 10mg of edrophonium chloride was injected prior to maximal static and dynamic exercise. Force output and surface EMG data were extracted from these experiments. In both experiments the results from statistical analysis of these variables reject the hypothesis that failure of neuromuscular transmission is a mechanism of fatigue under normal physiological
conditions in humans.

We defined fatigue as the inability of a muscle to maintain a desired force output. The pooled force records, represented as a percentage of the maximal peak tension, are shown in Figure 9. Although the raw data varied considerably between subjects, the normalized and pooled results clearly demonstrated linear relationships for all trials within a force range of 100% MVC to approximately 55% to 60% MVC. The lack of experiments in which dynamic protocols have been used leaves little flexibility for comparison of our dynamic trials to standards. The static trials on the other hand compare quite well with the fatigue curves from experiments using maximal quadriceps exercise, and with others using different muscle groups (Asmussen, 1979, Mills, 1982, and Stevens and Taylor, 1972). For the most part a linear relationship dominates the fatigue curves as illustrated in Figure 9. The rate over the linear portion of the curves in experiment one are within the range reported for MVC of the quadriceps. The values reported by Bigland-Ritchie (1981) are between 50%-70% MVC/minute. For the dynamic trials we calculated an average fatigue rate of about 66%/minute, while for the static condition a slower rate of about 50%/minute was observed. Since the subjects were pre-fatigued in experiment two the fatigue rates were much faster; approximately 101% MVC/minute and 60% MVC/minute for dynamic and static exercise respectively (Figure 13). When the force in experiment two, for both static and dynamic exercise, fell to approximately 60% MVC a nonlinear phase was observed (Figure 13). Stevens and Taylor’s (1972) data from the first dorsal interosseous also revealed a non-linear phase, as did Merton (1954) with adductor pollicis and Komi (1983) using the quadriceps. This trend was not as discrete in Experiment 1, but was comparable to Bigland-Ritchie’s (1979) experiments with the quadriceps, and with Hultman (1983) who stimulated the
quadriceps maximally. The non-linear phase observed in our experiments can be explained partially by subject atricia. Since our subjects fatigued at different rates, and the fatigue protocol was based on a defined level of fatigue rather than time, the number of subjects contributing to the force curve becomes smaller with time. As such, if a subject drops out the average force should theoretically rise. Evidence to support this comes from the dynamic pre-trials of experiment two (Figure 12), which illustrate almost a perfectly linear relationship from 100% MVC to 40% MVC. Observation of the individual force curves of certain subjects, however, show a definite non-linear phase where the above explanation can not apply. On a physiological basis synchronization of motor units, which has been shown during prolonged exercise (Burke, 1981, and Bigland-Ritchie 1981), could lead to a slowing of the rate of fatigue. Also, as fatigue progresses force output is dependant more and more upon the FR and S type units that fatigue at a lesser rate (Burke, 1981).

Preliminary testing of the experimental protocols revealed that when the subjects were asked to produce MVC's the peak tension was never attainable before the first 3-6 seconds of either dynamic or static exercise. With this observation we instructed the subjects in experiment one to produce three "build-up" contractions (as described in the methods), which was followed by maximal effort for the duration of the exercise. In experiment one fatigue then progressed as described above. For experiment two the subjects were not instructed to produce the "build-up" contractions, rather they were asked to exercise maximally exactly two minutes after the injections. Figure 13a and Figure 13b show that for both the static and dynamic trials there was always a short time delay before peak tension was reached. For the dynamic trials the time delay was the same for both the drug and control conditions, averaging 3.6
seconds. Out of the 24 trials, 12 subjects produced their peak tension on the third contraction, 7 on the second, 3 on the fourth, and 1 on the first and fifth contractions. In other studies using humans, where maximal voluntary effort was required, this trend has not been reported. However, records of maximal twitch tension in isolated motor units of the cat demonstrate posttetanic potentiation (PTP) and/or postactivation potentiation (PAP) (Burke, 1976, 1981). With both PTP and PAP the evoked twitch tensions can be enhanced by modulation of the activation history of the preparation. For PTP, the twitch recorded shortly after a tetanizing train of activation is usually of greater amplitude than the twitch recorded prior to tetani (Burke, 1981). For PAP, the second twitch in a train of pulses can be enhanced to amplitudes much higher than the first by adjusting the interpulse interval to an appropriate latency (for the cat hind limb motor unit an interpulse interval of 5-10 msec produces this effect [Burke, 1976]). Burke (1981) suggests that PTP and PAP are an inherent property of the muscle fibre. While this may be the case, the observations of the present study give some clues of a possible central origin. This conclusion is based on: (i) the fact that the dynamic pre-trials of experiment two showed the same trend with an average time to peak tension of 2.1 seconds, which suggests that the muscle responded in a similar fashion to different activation histories, and (ii) the EMG showed the same pattern as the force, as illustrated in figures 15a to 15b. The EMG is determined by motor unit recruitment and the rate and pattern at which the motor units fire. It is possible that any three or all of these variables reach an optimal level at some time after initial activation. This neurogenic theory is somewhat supported by a recent study by Suzuki et al (1988), in which single motor unit firing frequencies were increased subsequent to conditioning muscular contractions of 25% and 50% MVC. There was also evidence that the conditioning contractions lowered recruitment thresholds. Since our results show that force
output so closely mimics the EMG over the first few seconds of MVC, neurogenic potentiation is an attractive hypothesis, but certainly requires further investigation.

The peak tensions were calculated for each trial and compared in experiment one to ascertain whether the knee extensors were fully activated. As stated above, maximal force output is initially dependant upon recruitment of all motor units and the optimal firing rate and pattern of those units. From work by Grimby (1981), and Bigland-Ritchie (1981) it was assumed for these experiments that maximal motor unit recruitment was attainable for voluntary knee extension. If this is true, the only way to enhance force output is through the modulation of firing rate and firing pattern. The mechanism by which a change in firing rate can effect force output is through the enhancement of transmission. In order for this to occur more transmitter must be released per unit time. This is essentially the mechanism by which edrophonium chloride restores force generation in myasthenic patients, by allowing more Ach to reside in the synaptic cleft producing effects equivalent to excessive stimulation. Assuming in the present experiments that all of the motor units are recruited, the presence of edrophonium chloride should theoretically simulate optimal firing rate and to ensure maximal activation of the knee extensors.

There were no significant differences observed for the peak tensions between the drug and control conditions for both the static and dynamic exercise (Figure 10 & 14). Therefore under the conditions of these experiments the peak tensions represent the knee extensors in a fully, voluntarily, activated state. This conclusion was further supported by the observation that in over the 96 trials of experiment one and two, there were only five trials in which a subject produced a contraction that was greater than a prior contraction once the peak tension
was reached). This condition was predominantly observed during the static bouts (occurring only once during dynamic trials) of exercise where concentration was reported by most subjects to be more difficult. Bigland-Ritchie (1979) and Grimby (1981) observed similar periods of "extra effort" (see introduction page 10), in which peak tensions were greater at various points along the fatigue curve. They concluded a centrally fatiguing mechanism, however, it is our opinion that these studies did not provide sufficient evidence to support this claim (see page 11 of introduction). It is more likely that the subjects initially failed to fully activate all motor units through lack of motivation or experience (Bigland-Ritchie, 1984).

The values for the peak tensions, in newton-meters, are shown in Table 1 & 2 where a two fold difference in force generation between the static trials and dynamic trials was observed. The greater forces produced statically could be predicted from the force-velocity relationship, however it was unexpected that the rate of fatigue would be faster with dynamic exercise, since dynamically generated forces are smaller and the knee extensors are provided a rest period (where blood flow is restored to the muscles) during flexion. The unexpected results are not easily explained since most of the studies on muscle fatigue have been conducted using static protocols, and the existing dynamic protocols are not comparable to the static. One possible explanation may be related to the energy expenditure that is required for the continuous forming and breaking of the acto-myosin bonds during dynamic exercise, however, there is no experimental evidence to support this speculation.

For experiment one the peak tensions represented the absolute maximal voluntary force attainable by the subjects. Experiment two was designed to create a situation where the subjects maximal voluntary force was reduced relative to their absolute maximum. This is the condition that myasthenic patients
are faced with due to neuromuscular block. These patients are only able to
regain the ability to maximally activate their skeletal muscles with presence of
anticholinesterases. By prefatigueing the subjects with 40 maximal dynamic
contractions prior to the injection, this physical symptom of myasthenia gravis
was simulated. Subsequent to this procedure the drug or control was injected,
followed a minute later by maximal dynamic or static exercise. The peak
tensions (Table 2) were about 25% and 15% (dynamic and static respectively)
lower than the values calculated from experiment one (Table 1). It was
postulated that if this loss in force generating capacity was due in whole, or in
part, to failure of transmission processes, the presence of edrophonium chloride
would restore the peak tensions toward values obtained in experiment one. There
was no evidence to show that the peak tensions for any of the static or
dynamic drug trials in experiment two were enhanced or statistically different
from the control trials. Thus the loss in force generating capacity created in
experiment two can not be attributed to transmission failure (at least with
respect to transmission processes proximal to the end plate).

It was stated in the hypotheses that to produce force, an EMG signal must
be present, however, to have EMG present does not mean that force must
follow. The point being that fatigue is characterized by a number of different
features (some of which have already been described), in which one process may
involve the failure of transmission while another may not. For this reason we
analyzed the effect of edrophonium chloride on four other force variables
representing different phases of fatigue, as well as the integrated EMG (IEMG).
We defined the time to onset (TTO) of fatigue as the last point where the
subjects force was 90% MVC. This variable has not been previously described,
however, it was selected for comparison because the early phases of fatigue are
likely to differ from the later stages. This speculation was based on the evidence that the FF-type fibres that are recruited for large forces and fatigue much faster than the S-type fibres. Stimulation experiments in animals (Pagala, 1984, Clamann and Robinson, 1985, Kugelberg and Lindegren, 1979, and Enoka et al, 1987) have suggested that the FF-type fibres are more susceptible to transmission failure. If this were the case in our experiments the TTO would be expected to increase in the presence of edrophonium chloride. Figure 10 and 14 illustrates that the drug did not have an effect on TTO under dynamic or static conditions for both experiment one and two rejecting the above statement. Considering the observations of the authors above, who demonstrated that FF-type fibres are more prone to transmission failure than the fatigue resistant fibres, several attempts were made to categorize the subjects into fatigue sensitive group. This was done to investigate whether individual variations in the subjects may have masked a drug effect, which might have occurred if the drug effected certain subjects, and had no effect on others. There were only three subjects where a drug effect was shown, however this was never consistent for all trials for that subject, rejecting the possibility edrophonium chloride had an effect on certain individuals with common neuromuscular characteristics.

The later stages of fatigue were investigated with analysis of the latency from 100% MVC to a pre-defined percentage of MVC (TTF). For the dynamic exercise the pre-defined value was 55% MVC and for the static condition 60% MVC was used. These values generally occurred close to or within the non-linear portion of the fatigue curve. By subtracting TTO from TTF a value defined as the time course (TC) of fatigue was calculated. Depending on how edrophonium chloride effected TTO and TTF, three outcomes were postulated for TC: (1) The TC was significantly reduced after edrophonium chloride as a result of an
increase in TTO and no change in TTF. For this to occur the drug would have enabled the subjects to maintain force output above 90% MVC, which would have supported the findings from selected animal studies that the FF-type fibres are more susceptible to transmission failure. Since we have already disclosed no change in TTO this hypothesis was rejected. (2) The TC was significantly increased due to an increase in TTF with no change in TTO. Under these conditions edrophonium chloride was predicted to either restore activity in fibres that have dropped out or prolong the activity of the fibres still active. Figure 10 & 14 shows the TTF and TC data and it is clear from this and statistical analysis that the drug had no effect on these variables. The above findings also reject our third prediction for TC, that both TTO and TTF increase resulting in no change in TC.

All of the above data compared latency values derived from constant fatigue levels. Thus for completeness a final comparison was computed at a constant time during fatigue. For this test the percent value of the MVC (TS% MVC) was calculated at a maximal time common to both the drug and control condition, on a subject to subject basis. Figures 10 & 14 illustrate the results of the TS% MVC data and support the findings described earlier that show no effect of the drug on fatigue. Thus the force data presented in these experiments clearly reject the hypothesis that transmission is impaired during fatigue of the knee extensors from MVC.

As stated earlier, the EMG signal is not dependant upon force, thus changes in the EMG may occur without any observable changes in force. In fact Clamann (1985) observed a 75% reduction of IEMG and no loss in force with high frequency-stimulation of S-type motor units isolated from the cat hindlimb. In humans fatigue experiments, others have reported a reduction in evoked M-wave
amplitude and/or area (Stevens and Taylor, 1972, Bellemare and Garzaniti, 1988), while still others have shown no change or even a facilitation of the M-wave during MVC (Merton, 1954, Bigland-Ritchie, 1979, 1982). Thus it was quite possible in the present experiments that EMG might increase in response to edrophonium chloride with no change in force. Under such conditions fatigue could then be attributed to mechanisms distal to the sarcolemma.

In both Experiments the raw EMG signal was extracted from the surface of the vastus lateralis and the rectus femoris. The amplitude of the rectified signal ranged between 5 mV and 12 mV with the values being marginally higher over the vastus lateralis. The rectified signal was digitized, integrated, then plotted against time as a percentage of the maximal value. For statistical analysis a second integration was performed from these curves leaving a single value representative of the overall EMG activity of the selected muscle. The EMG (mean values represented as a percentage of the maximum) vs time curves for all conditions of experiment one and two are shown in figures 11 & 15. There does not appear to be any differences in the pattern of electrical fatigue from the different muscle groups, which suggests that the VL and RF contribute synergistically to force output, and are affected by fatigue in a similar way.

Although surface EMG correlates well with maximal voluntary force, it cannot provide specific information about mechanisms of fatigue. It would therefore be unreliable to extract anything other than subjective speculations from the data presented in these experiments. The IEMG data of experiment one demonstrates essentially the same fatigue pattern as force, with exception of the first few 3-5 seconds of activation. During the early stages of exercise the IEMG was never maximal at the time corresponding to the peak tensions recorded. The delay observed in the peak IEMG recorded is probably not an artifact since the
same trend can be seen in EMG records of Bigland-Ritchie (1978) and of Kranz (1985). Since it was not a topic of their investigation, these authors did not address this observation, however there are some properties of the motor unit that may explain the uncoupling of the force and EMG. Most of the EMG over the early stages of MVC is dominated by the activity from the large FF-type units. Associated with fatigue of these motor units is, a slowing of sarcolemmal conduction velocity (Milner-Brown, 1986, and Bigland-Ritchie, 1981), a downward shift in the power spectrum (Mills, 1982, and Viitasalo and Komi, 1971), and motor unit synchronization (Lippond, 1962, and Bigland-Ritchie, 1981, 1984), all of which can alter the appearance of the EMG signal without influencing force. Once the IEMG reached a maximal value it fell off at a slower rate than force and at various points along the later stages of the curve sporadic fluctuations in the IEMG occurred that did not appear to be correlated to force output.

Experiment two yielded much the same results, however it was interesting that without the "build-up" contractions of this experiment the IEMG matched the early stages of force output. This was particularly obvious with dynamic exercise where the relative magnitude of the IEMG incremented exactly the same as force for the first three contractions. Once peak tension was reached the IEMG signal became more like experiment one characterized by a slow rate of failure superimposed with radical fluctuations.

Tables 1 & 2 show the total IEMG values along with the standard deviations. The error values recorded between the twelve subjects are clearly large enough to raise questions as to the possibility that a real drug effect may have been masked. This is probably not the case, however, since observation of the subjects individual data does not reveal any clues to support a drug effect.
Statistical analysis of the integrated-IEMG were consistent with the force analysis revealing that edrophonium chloride had no effect on electrical failure during fatigue.

0.13 Summary And Conclusions

Although transmission failure is readily demonstrated with high frequency stimulation in vitro, and in humans, with myasthenia gravis, its presence during repetitive activity in normal individuals has not been convincingly demonstrated. Studies completed to date have made controversial conclusions about transmission mechanisms through observations of surface EMG trends of an active muscle. The present study was unique in that transmission processes were pharmacologically manipulated to ensure optimal levels of acetylcholine in the synaptic cleft during fatiguing exercise. The results of this investigation support the conclusions of Bigland-Ritchie, and others, that transmission mechanisms are intact and are not a limiting factor in force production with fatigue from MVC. From this it follows that fatigue must involve failure of events distal to the endplate, and/or events that effect motor unit activation. At present mechanisms supporting the former dominate the field. This does not necessarily mean that the events limiting force output predominate distal to the endplate. The large volume of data correlating biochemical, metabolic, and mechanical precursors to fatigue may simply reflect the state of technology. Advanced biochemical techniques have facilitated research into peripheral fatigue, while technical advancements in neurophysiology still limit investigation into central fatigue of the intact organism. Force production involves the interaction of many events in the neuromuscular system, thus it is predictable that as many processes may influence the course of force loss. The simple definition of fatigue (the inability
of the neuromuscular system to maintain a desired force output) can basically be applied to all exercise protocols, however, the causes of fatigue under different exercise conditions are likely to be shown not consistent. The system can be fatigued under static or dynamic conditions, through submaximal or maximal voluntary activation, or with the use of stimulation techniques. Each of these may involve different interactions of the motor unit types, and it has been well established that FF, FR, and S-type units differ significantly with their contribution to force production. Therefore, we reject the possibility of transmission failure during maximal static and dynamic knee extension in humans, but we can not apply these conclusions to fatigue induced with different exercise protocols, or with muscles of a different composition and function.
PART F
APPENDIX
Figure 6. Test for A/D conversion repeatability over four trials using the same section of FM tape. A. Dynamic test, B. Static test.
Figure 7. Example of manual digitization process of force data extracted from one dynamic trial.
#include <labhead.h>
int gain[16]= { 3,3,0,0,0,0,0,0,0,0,0,0,0,0,0,0 }; /* channel 0 (rectus femoris) +1.25 volts code 0 */
int channel[2]={0,1}; /* channel 1 (vasos femoros) +1.25 volts code 0 */
int emgdata0; int emgdata1;

int kickindex; /* index to timer array, begin of each kick */

int intindex; /* index to number of integrals, theoretically (?) */

unsigned kicktimes[50]; /* this will be indexed by kickindex */
int integrals[50][2]; /* indexed by intindex; can only be max 50 kicks */
char string[20],schar[10]; /* keyboard data entry area */

long triglevel; /* trigger level of emg to switch onquisition */
long hysteresis; /* to hold inquisition loop longer */
long totalemg0; /* integral of 1/10 sec data segments */
long totalemg1; /* integral of 1/10 sec data segments chan 1 */
long tempemg;

newrun = 0; /* unless otherwise, dont execute main loop */
printf("\n\nLabpac EMG DYNAMIC program\n");
printf("\n\nCode by Rob Taylor and Greg Jensen\n");
printf("\n\nRectus femoros chan 0, + 1.25 volts\n");
printf("nVastus femorus chan 1, + 1.25 volts");
printf("nChannel 1 will trigger data acquisition");
printf("nThe program Labpcac.com must be run before this program!");
nrun=1;
LP (RESET); /* Reset labpcac arrays */
LP (AIINIT, ATOD, 16, 0, gain); /* Initialize A/D conversion */
LP (TIINIT,TIMER);
while (newrun) {
  intindex=0;
  printf("n
Enter trig level: ");
  scanf("%d", &triglevel);
  printf("n
Enter hysteresis level: ");
  scanf("%d", &hysteresis);
  LP (TIST,5,14,2); /* 500 HZ main timer */
  LP (TIST,1,0,10); /* 50 HZ timer */
  LP (TIST,2,0,5); /* 10 HZ timer */
  LP (TIST,3,0,0); /* keep track of timer 2 pulses */
  kickindex =0;
  while (lkbhut()) { /* Main acquisition loop */
    integrals [intindex][0]=0; integrals [intindex][1]= 0;
    while(totalemg1<triglevel) { /* wait for begin pulse loop */
      ifdef DEBUG
      printf("nWAIT");
      endif DEBUG
      emgdata1 = LP(AIRAW, 1); /* read muscle */
      emgdata0 = LP(AIRAW,0); /* read muscle */
      totemg1 = totemg1 + emgdata1; /* Sum muscle */
      totemg0 = totemg0 + emgdata0;
    }
  }
  } /* End wait pulse loop */
  kicktimes[kickindex] = LP(TIRAW,3); /* Read begin kick time */
#endif DEBUG
printf("nKicktime: %d",kicktimes[kickindex]);
```c
#endif
kickindex ++;
tempreg = 20000; /* Force entry into while loop */
while (tempreg >= triglevel-hysteresis) {
    /* Start aquire loop */

#endif DEBUG
<end:
printf("\nAQUIRE ");
#endif

/* move up 1/10 sec on array index */
LP(TIST,5,14,2); /* Re-Start timer at 500 Hz. */
tempreg = 0;
for (i=0; i<50; ++i) {
    LP(TISTAT,5,i); /* wait 1/500 second */
    emgdata0 = LP(AIRAW, 0); /* read muscles */
    emgdata1 = LP(AIRAW, 1);
    totalemg0 = totalemg0 + emgdata0;
    totalemg1 = totalemg1 + emgdata1; /* sum 1/10 sec */
    tempreg = tempreg + emgdata1;
}
} /* End data aquire loop */
integrals [intindex][0] = ((int)(totalemg0/100));
integrals [intindex][1] = ((int)(totalemg1/100));
totalemg0 = 0;
totalemg1 = 0;
++ intindex;
} /* End main loop */
#endif DEBUG

printf("AEND DATA AQUISITION\n");
#endif
printf("Screen dump? (y/n) ");
scanf("%s",schar);
if (!strcmp(schar, "y")) {
    printf("Screen dump? (y/n) ");
    printf("\n--- integrals ---");
    printf("\nKick Time Channel 0 Channel 1 ");
    for(i=0; i<kickindex;++i) {
        if(!kbbhit) break; /* kill screen dump if kbd hit */
        printf("% Ohm -8d % Ohm -8d % Ohm -8d",i,kicktimes[i],integrals[i][0],
                integrals[i][1]);
```

```c

```
long temp0, temp1;
int kick;
int integrals[40][2];
for (i = 0; i < 12000; ++i) {/* initialize data array */
    emgdata[i][0] = 0;
    emgdata[i][1] = 0;
}

printf(" Labpac EMG STATIC program");
printf(" Code by Rob Taylor and Greg Jensen");
printf(" Rectus femoros 0, + 1.25 volts");
printf(" Vastus femoros 1, + 1.25 volts");
printf(" The program Labpac.com must be run before this program!");
printf(" This program saves one out of every three seconds of data");
printf(" Sample data? (y/n) ");
scanf(%s, achar);
newrun = 0;
if (strcmp(achar, "y"))
    newrun = 1;
labpac(RESET);    /* Reset labpac arrays */
labpac(DINIT, ATOD, 16, 0, gain); /* Initialize A/D conversion */
while (newrun) {
    arrayindex = 0;
    kick = 0;
    printf(" Sampling inputs ");
    while ((kbhit()) && (arrayindex < 12000)) {
        printf(" Saving starting at array index: ", arrayindex);
        labpac(TINIT, TIMER);    /* Initialize timers */
        labpac(TIST, 5, 14, 2);    /* start timer at 500 hz */
        labpac(TIST, 1, 0, 0);    /* timer 1 500 hz */
        temp0 = 0;
        temp1 = 0;
        for (i = 0; i < 500; ++i) {/* Read one second loop */
            labpac(TISTAT, 1, 1);    /* wait 1/500 second */
            emgdata[i + arrayindex][0] = labpac(AIRAW, 0);    /* Save data in arrays */
        } /* Read one second loop */
        arrayindex++;
    } /* Sampling inputs */
} /* while (newrun) */

/* Rest of the code... */
emgdata [i+arrayindex][1] = labpac(ARAW, i);

temp0 = temp0+emgdata [i+arrayindex][0];
temp1 = temp1+emgdata [i+arrayindex][1];

} // Print out integrals
integrals[kick][0] = temp0/100;
integrals[kick][1] = temp1/100;
++kick;
i...

emgdata[i+arrayindex][0] = 9999; /* Mark end of 1 second segment */
emgdata[i-arrayindex][1] = 9999;

arrayindex = arrayindex+500;
labpac(TINIT,TIMER); /* Initialize timers */
labpac(TIST, 5, 14,2); /* start timer at 500 hz */
labpac(TIST, 1, 0, 0); /* timer 1 = 500 hz */
printf("\n\nTwo second wait ");

for(i=0; i<1000; ++i) { /* Wait two second loop */
labpac(TISTAT,1,i); /* wait 1/500 second */
}
/* end main acquire loop */
printf("\n\nScreen dump? (y/n) ");
scanf("%s",achar);
if (!(strcmp(achar , "y"))) {
    printf("\n\n Integrals \n");
    printf("\n\nKick Channel 1 Channel 2\n");
print("------------------------\n");

for(i=0; i<kick; ++i) {
    if(kbhit()) break; /*kill screen dump if kbd hit */
    printf("%-4d %-8d %-16d\n",i, integrals[i][0],integrals[i][1]);
}
}
printf("\nSave data? (y/n) ");
scanf("%s",achar);
if (!(strcmp(achar , "y"))) {
    printf("\nFilename: ");
    scanf("%s",string);

    file = labpac(OPEN; string);
    printf("\nWriting to file %s",string);

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LP(WRITE, file, kick, 2, channel, integrals);
labpac(CLOSE, file);

} for(i=0; i < 12000; ++i) { /* initialize data array
   emgdata[i][0] = 0;
   
   emgdata[i][1] = 0;

} newrun = 0;

printf("Another run? (y/n) ");
scanf("%s", achar);

if (!strcmp(achar, "y"))
   newrun = 1;
} /* end while newrun */
} /* end main */
PART G

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