A NEW METHOD FOR ESTIMATING THE NUMBER OF MOTOR UNITS IN A MUSCLE

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Abstract
A new method termed MUESA - Motor Unit Estimation based on Stochastic Activation - was developed for estimating the number of motor units in peripheral nerve/muscle systems. What distinguishes MUESA from other estimation methods is the manner in which it deals with "alternation" or probabilistic motor unit activation. Because of "alternation", incremental increases in the observed muscle potentials often cannot be interpreted in terms of the successive activation of single motor units. With MUESA, we introduce a method that interprets the muscle potentials in the context of a probabilistic activation framework.

In the MUESA method, the nerve is subjected to a number of constant-intensity stimulus trains, and the resultant muscle response sequences are decomposed into their constituent motor unit action potentials. In general, if a stimulus train results in the probabilistic activation of n motor units, we can expect to see up to 2^n different potentials, with each potential representing a unique combination of active and/or inactive motor units. If all 2^n potentials are indeed observed, the decomposition of the observed potential sequence into its constituent motor unit action potentials is trivial. For the majority of the cases in which the number of observed potentials is not an integer power of 2, we have developed a novel decomposition method based on the analysis of the relative firing rates of the motor units. MUESA was evaluated by examining the estimates obtained from both control and neurogenic subjects.

We also examined a number of alternative estimation strategies that do not rely on sampling procedures used in all methods published to date. These alternative methods hold the promise of avoiding the inherent error associated with sampling.
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<td>EMG</td>
<td>Electromyography</td>
</tr>
<tr>
<td>ENS</td>
<td>Electrical Nerve Stimulation</td>
</tr>
<tr>
<td>ICT</td>
<td>Increment Counting Technique</td>
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<td>MU</td>
<td>Motor Unit</td>
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<td>M-Wave</td>
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<td>NFAP</td>
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1. INTRODUCTION

1.1 Definition of a Motor Unit

Skeletal muscle fibers are organized into functional groups termed motor units (MUs), with each MU consisting of a motor neuron (also termed motoneuron) and all of the muscle fibers innervated by it (Figure 1). This imposes a structure in which the constituent muscle fibers for any given MU can not act independently of one another.

MUs play an integral role in muscle physiology, with all of the varied reflex and voluntary muscle contractions being achieved by different combinations of active MUs. MUs adapt to changes in usage (Fuglsang-Frederiksen and Scheel, 1978; Edstrom and Grimby, 1986), are affected by aging (Campbell et al., 1973; Brown et al., 1988) and by various diseases (Walton, 1974). Given their central role, it is not surprising that the development of an accurate and reliable technique to evaluate the number of MUs in a muscle would yield numerous clinical benefits. As Brown (1984) states, "one of the most desirable indices investigators would like to measure in peripheral nerve and to some extent muscle diseases is the number of MUs present". Specifically, such quantification would:

1. permit the accurate and objective monitoring of the natural history of the disease,
2. permit the evaluation of different therapies in a quantifiable manner, and
3. facilitate the early detection of muscle denervation which is manifested by a decrease in the number of motor units.

Over the course of the last several years, there has been a renewed interest in developing methods aimed at quantifying the number of motor units in muscles (Slawnych et al., 1987; Jasechko and deBruin, 1987; Brown et al., 1988; Cavasin et al., 1988; deKoning et al., 1988; Daube 1989). In this thesis, we present our investigation of this problem.
Figure 1: A schematic illustration of a motor unit, which consists of two integral components: the motor neuron and muscle fibers that it innervates. The muscle fibers of any given MU are generally interspersed with muscle fibers from different MUs.
1.2 Terminological Considerations

To date, all of the published methods for quantifying the number of MUs in a muscle have adopted a sampling procedure in which the properties of only a relatively small number of MUs are examined. As such, the numbers derived by these techniques are associated with an inherent sampling error. Hence we term the numbers derived by these methods 
*MU estimates*. The term *MU count* is also commonly used and should be interpreted as being the same as an MU estimate.

1.3 Basis for MU Quantification Methods

In response to activation by the neural input, the muscle fiber produces a mechanical response in the form of a contraction. This mechanical response is initiated by a wave of electrical depolarization/repolarization, termed the action potential, that propagates along the fiber membrane. The mechanical response associated with the activation of a single MU is termed a twitch. The associated electrical activity is termed a motor unit action potential [MUAP]. MUAPs play a central role in electrodiagnostic methods aimed at determining the physiological and functional status of muscles. Specifically, there are two major approaches to the electrodiagnostic examination of the motor system. One involves the evaluation of the signals produced in response to voluntary muscle contractions. At small contraction levels, individual MUAPs can be readily identified. However, these early-recruited MUs are not representative of the entire MU population (Milner-Brown et al., 1973; Kadrie et al., 1976). Stronger contractions produce more complex signals, or interference patterns, which represent the asynchronous activity of many different MUs (Figure 2a). Interference patterns are generally classified in terms of parameters such as mean amplitude and "turns" density which have little direct relationship to the number of constituent MUs in the muscle (c.f. Gilai, 1989). Some of the newer techniques move away from these types of parameters and actually extract MUAPs from the interference pattern, which then can be used to estimate the number of MUs in the muscle (Brown et al., 1988; deKoning et al., 1988).
The second approach by-passes the higher centers involved in voluntary activation by electrically stimulating the nerve innervating the muscle. In this case, the external electrical stimuli act to synchronize the activation of the MUs, yielding potentials that are typically biphasic or triphasic in nature (Figure 2b). These evoked potentials are the product of two adjoint properties of the electrically stimulated nerve/muscle system:

1. the sizes of the individual MUs in terms of the number of constituent muscle fibers, and
2. the number of MUs activated by the stimulus pulse.

We cannot control the first property since it is inherent to the muscle itself. However, the second property can be controlled by carefully grading the intensity of the stimulus. It is this potential ability to control the number of active MUs that first inspired MU estimation techniques (McComas et al., 1971; Brown, 1972). The method that has received the most attention is the increment-counting technique, developed by McComas and colleagues (1971) for estimating the number of motor units in small muscles such as the extensor digitorum brevis. However, despite a number of modifications (Ballantyne and Hansen, 1974; Panayiotopoulos et al., 1974; Milner-Brown and Brown, 1976; Brown and Milner Brown, 1976; Jasechko and deBruin, 1987), a number of theoretical and practical limitations remain.
Figure 2 Comparison of the two different ways in which MUs can be activated. a) Signal produced in response to a voluntary muscle contraction, in which case we have the asynchronous activation of MUs. b) The signal produced in response to a stimulus train which results in the synchronous activation of MUs (the narrow spike preceding the main signal represents the response of the amplifier to the stimulus pulse and is termed the stimulus artifact).
1.4 Thesis Organization

We begin by reviewing the published estimation methods in Chapter 2. As stated in Section 1.2, the estimates obtained using these methods are associated with an inherent sampling error. We developed a computer model of the sampling procedure to investigate the magnitude of this error. The results of this investigation are also presented in this chapter. In Chapter 3, we introduce the estimation method that we have developed, termed MUESA, which is an acronym for Motor Unit Estimation based on the Stochastic Activation of motor neurons. As the name suggests, MUESA capitalizes on the probabilistic manner in which MUs are activated in response to external electrical stimulation. Hence we devote the remainder of Chapter 3 to the discussion of this issue. In Chapter 4, we discuss the methods we use to classify the recorded muscle potentials. The details concerning the implementation of MUESA are discussed in Chapter 5. MU estimates obtained from both normal and diseased muscles are then presented in Chapter 6. In Chapter 7, we explore alternative estimation strategies that move away from the sampling procedure inherent in all of the published methods. To our knowledge, this work represents the first time that any such alternative methods have been investigated. We then conclude with a discussion in Chapter 8.
2. REVIEW OF PUBLISHED MU ESTIMATION METHODS

2.1 Formulation of the Estimation Procedure

All of the published MU estimation methods are based on a sampling procedure in which two pieces of information are required:

1. the summated response of all of the MUs, and
2. the mean MU response, which is usually calculated by examining a relatively small number of MUs which are assumed to be representative of the entire MU population.

These responses are generally measured in terms of the amplitudes or areas of their associated potential waveforms, although tension measurements have also been employed (Stein and Yang, 1990).

Given the summated and mean responses, the MU estimate (N) is simply calculated as follows:

\[ N = \frac{M}{m} \]  \hspace{1cm} [1]

where \( M \) is the amplitude (or corresponding area or tension) of the summated response and \( m \) is mean MUAP amplitude (or corresponding area or tension). Since the calculation of the motor unit estimate is the same regardless of which measure is employed, the remaining discussion will assume that amplitude values are used.

The summated response is easily obtained by electrically stimulating the nerve at supra-maximal levels. This results in the near-synchronous activation of the entire MU pool\(^1\). The associated electrical response, which is termed the maximal evoked potential or M-Wave, is generally

\(^1\)The activation is not completely synchronous since the all of the motor neurons do not conduct action potentials at the same velocity.
biphasic in nature (Figure 3). The acquisition of the representative MU sample, on the other hand, is not as straightforward, and is discussed below.

2.2 Obtaining the Representative Sample

The acquisition of the representative MU sample has been approached in two different ways. A number of investigators have employed external electrical stimulation to obtain the MUs (McComas et al., 1971; Brown 1972; Panayiotopoulos et al., 1974; Ballantyne and Hansen, 1974; Miner-Brown and Brown, 1976; Jasechko and deBruin, 1987; Daube 1988; Stein and Yang, 1990). More recently, investigators have started to employ techniques based on voluntary muscle contraction (Brown et al., 1988; deKoning et al., 1988; Barkhaus et al., 1990). We have termed these the Electrical Nerve Stimulation [ENS] and Voluntary Muscle Activation [VMA] methods, respectively. The basic premise behind these methods is discussed below. A comprehensive review can be found in recent papers by Slawnych et al. (1990) and McComas (1991).

2.2.1 ENS Methods

McComas and colleagues (1971) pioneered the field of MU estimation by developing an approach based on the delivery of a carefully graded sequence of stimulus pulses. This results in successive activation of individual MUs, with the activation of each new MU being associated with an incremental increase in the recorded compound potential (Figure 4). This procedure of increasing the stimulus intensity and observing the resultant incremental responses is continued until approximately ten MUs have been activated. The mean MUAP amplitude is then calculated as follows:

$$ m = \frac{\text{Amplitude of the Largest Observed Compound Potential}}{N_{\text{MU}}} $$

[2]
where \( N_{\text{MU}} \) is the number of constituent MUs. The nerve to the muscle is then supra-maximally stimulated, yielding the maximal response. The motor unit estimate [MUE] is then calculated using equation [1]. This approach has been termed the increment counting technique [ICT].

A number of key assumptions are associated with the ICT:

1. The incremental increases in the recorded potentials correspond to the additional activation of single MUs,
2. The MUAPs used to calculate the mean MUAP are representative of the entire MU population, and
3. the recorded electrical activity is exclusively derived from the muscle under investigation.

The validity of these assumptions has been challenged (c.f. Slawnych et al., 1990). Additionally, the method has come under criticism due to some of the results obtained with it. Specifically, McComas and colleagues found reduced MUEs in subjects with myasthenia gravis (McComas et al., 1971), and Duchenne and limb-girdle muscular dystrophies (McComas et al., 1971d; Sica and McComas, 1971), which suggests a possible neurogenic basis for these disorders. This is not universally accepted. In an effort to remedy some of its shortcomings, several researchers have introduced modifications to the method. These modifications are discussed in Appendix 1. Appendix 2 provides a comprehensive listing of MU estimates obtained by various investigators.
Figure 3: The M-Wave response which represents the near-synchronous activation of the entire MU pool. It is obtained by supra-maximally stimulating the nerve innervating the muscle.

Figure 4: Potentials observed in response to graded electrical nerve stimulation. The increment-counting technique assumes that each successive potential represents the activation of a new MU.
2.2.2 VMA Methods

Brown et al. (1988) and deKoning et al. (1988) have recently developed estimation methods based on the analysis of muscle potentials generated in response to voluntary muscle contractions. These methods employ the spike-triggered averaging and Macro-EMG techniques, respectively.

The spike-triggered averaging technique encompasses both surface and intramuscular recordings and proceeds as follows. A single-fiber electrode is used to selectively record the potential of an individual muscle fiber. This potential serves as a trigger source from which to average the potential of a single MU from the summated, surface recorded muscle activity during a voluntary, isometric muscle contraction. However, the spike-triggered averaging technique does not, in itself, provide a MUE. Therefore, Brown et al. (1988) have extended the technique in the following manner. The above procedure is performed ten times with the single-fiber electrode being positioned at different locations, yielding 10 different MUAPs. The mean MUAP amplitude is calculated as follows:

\[
\begin{align*}
N_{MU} \sum_{i=1}^{N_{MU}} \text{Amplitude of the } i\text{th MUAP} \\
m = \frac{\text{Amplitude of the } i\text{th MUAP}}{N_{MU}}
\end{align*}
\]  

[3]

where \(N_{MU}\) represents the number of MUs in the sample. The maximal response is then obtained by supra-maximally stimulating the nerve, and the MUE is calculated using equation [1].

DeKoning et al. (1988) developed a similar method based on the Macro EMG technique, the major difference being that the recorded potentials are exclusively intramuscular. These potentials are recorded using a "macro" electrode consisting of a cannula surrounding a single-fiber electrode. On each insertion of the electrode, five macro MUAPs are recorded at slightly different depths in response to a small-to-moderate intensity voluntary isometric muscle
contraction. With the electrode in the same insertion and positioned at one of the previous depths, the macro M-Wave is then elicited by supra-maximally stimulating the nerve to the muscle. The number of MUs present in the vicinity of the insertion is given by the amplitude (or area) of the macro M-Wave divided by the amplitude (or area) of the mean macro MUAP. The above sequence of events is carried out at four different insertion sites. The mean number of MUs from these four insertions is then calculated and referred to as the Motor Unit Density [MUD]. Obviously, the macro EMG based method only provides estimates of the numbers of MUs within localized areas of the muscle.

Again, as with the ENS methods, these methods assume that the MU used to calculate the mean amplitude are representative of the entire MU population.

2.3 A Comparison of the Two Methods
2.3.1 Recruitment Biases

The type of MUs that are recruited by the VMA methods are governed by the size principle which states that the MUs are recruited in an orderly fashion from smallest to largest. (Henneman, 1974). Since the VMA methods recruit their MUs at low to moderate contraction levels, they are biased towards the recruitment of the smaller sized MUs, and hence will tend to over-estimate the number of MUs in the muscle.

The recruitment of MUs in response to external electrical stimulation is not as straightforward. In particular, there are a number of factors which influence the order in which MUs are activated, including the diameter of the nerve axon, the distance between the axon and the electrode, the conductive properties of the nerve and intermediate tissue, and the shape and duration of the stimulus pulse. In theory, with all other factors being equal, axons with the largest diameter should be preferentially activated (Rushton, 1951). However, this does not take into account the nonhomogeneous nature of the nerve and surrounding tissue. Kadrie et al. (1976) found that
weak electrical percutaneous stimulation was biased towards the activation of smaller MUs. McComas and colleagues (1974), on the other hand, found no evidence of this bias.

In comparing the two different ways of activating MUs, Brown and Milner-Brown (1976) and Kadrie et al. (1976) found that MUs activated in response to voluntary muscle contractions were larger than those activated using electrical stimulation. However, Stein and Yang (1990) found that the average sizes of the MUs obtained using the two different methods were not significantly different.

2.3.2 Linearity

In calculating the MU estimate using equation [1], it is assumed that the individual MUAPs summate in a linear fashion to yield the maximal evoked response. This translates to the following

\[ M(t) = \sum_{j=1}^{N} a_j(t) \]  [4]

where \( M(t) \) is the M-Wave response, \( a_j(t) \) is the \( j \)th MUAP and \( N \) is the number of MUs. However, the contribution of a particular MU to the compound muscle potential depends on a number of factors, including muscle conductivity. Hence, equation [4] should be re-written as follows

\[ M(t) = \sum_{j=1}^{N} w_j a_j(t) \]  [5]

where \( w_j \) is a weighting factor.

Parry et al. (1977) evaluated the influence of muscle conductivity on the MU estimates using both modeling and experimental studies. In particular, they modeled the muscle system in terms of a
number of parallel MUs. Each MU was represented as a series combination of a voltage source and a source conductance, and was switched into the circuit only when it was active. Using this model, they predicted that the contribution of any given MU to the recorded surface potential decreased as a function of the number of active MUs. That is, the individual MUAPs did not summate in a linear fashion to yield the M-wave potential. The model explained this non-linearity in terms of a progressive increase in muscle conductivity as increased numbers of MUs are activated. They also demonstrated this non-linearity experimentally by recording the contribution of a number of MUs at both low stimulation levels (at which only a few MUs are active) and at supra-maximal stimulation levels (at which the entire MU population is active). On average, Parry and colleagues found that the MUAP amplitudes decreased by 20%. Since the MU estimate is inversely proportional to the mean MU amplitude, this corresponds to a 12.5% overestimation of the number of MUs in the muscle. However, there may be a number of errors associated with their calculations. Firstly, there is some variability associated with the amplitude of the M-wave, and hence it is difficult to quantify the exact contribution of any given MU to the M-wave response (c.f. Section 5.4.1). Secondly, the model employed by Parry and colleagues grossly oversimplifies the actual muscle system. In particular, they treat each MU as a lumped element consisting of purely resistive impedances, and assume that all of the MUs are in parallel with one another. Using computer models, Gielen et al. (1986) and Roth et al. (1988) have shown that detailed anatomical parameters play an important role in the evolution of the recorded muscle potential and hence must be taken into account by any model of the muscle system. Interestingly, Roth and colleagues found that changes in the impedance of the muscle fiber membrane (and hence the conductivity of the MU) do not significantly influence the recorded potential. Therefore, at the present time, the role that muscle conductivity plays on the validity of the MU estimate remains uncertain.

It should be pointed out, however, that if the conductivity of the muscle system changes with the number of active MUs, it will influence the ENS and VMA methods in different ways. In particular,
the ENS approaches recruit their MUs when relatively few MUs are active, whereas the VMA MUs recruit their MUs under conditions in which more MUs are active.

2.3.3 Difficulties Associated with the Acquisition of the MU Sample

As stated, an assumption of the ENS methods is that each incremental increase in the recorded potential corresponds to the additional activity of a single MU. The validity of this assumption has been extensively scrutinized. Firstly, Milner-Brown and Brown (1976) have shown that under normal conditions, MUAPs can exhibit small changes in shape and amplitude. These fluctuations may be mistaken for new MU activity. The same also holds under pathological conditions. Stalberg and Ekstedt (1973) showed that after partial denervation, MUAPs can exhibit fluctuating amplitudes during the course of reorganization. Secondly, due to the probabilistic nature in which MUs are activated in response to external electrical stimulation, incremental increases in the recorded potentials may represent different combinations of intermittently active MUs. Specifically, the "all-or-none" nature of action potential propagation invites the view that there is a single stimulus intensity level at which an individual nerve fiber will always be activated. However, this is not the case. Each MU exhibits a stimulation range over which the probability of activation increases from 0 to 100% (Figure 5). This probabilistic activation can be partially attributed to small variations in the resting membrane potential, as shown in Figure 6 (Verveen 1969, 1974). In addition, there may also be some variability in the intensity of the stimulus current reaching the motor neurons due to such factors as pulsatile blood flow.
Figure 5: The probability of activating a single motor neuron as a function of stimulus intensity. The shaded area represents the stimulation range over which the motor neuron exhibits probabilistic activation.

Figure 6 Variations in the resting membrane potential as a function of the absolute resting membrane potential level (from Verveen, 1969).
Given that a stimulus intensity level intersects the activation ranges of a number of different MUs, repetitive stimulation will result in the observation of multiple responses corresponding to different combinations of these MUs. Hence, each successive incremental increase in the observed compound muscle potential will not necessarily correspond to the additional activation of a new MU.

It should be pointed out that in the original MU estimation method developed by McComas and colleagues (1971), the premise of probabilistic MU recruitment is not completely ignored. In particular, these investigators recognize that an "alternation" phenomenon can occur in which different MUs are alternatively activated. In fact, some of the newer methods based on the increment-counting technique now employ what have been termed "alternation" detection algorithms to deal with the probabilistic recruitment issue.

It should also be noted that the first few MUs are usually activated independently of one another. Therefore, in this case, the incremental increases in the recorded potential do correspond to the successive activation of individual MUs. Kadrie et al. (1976) incorporated this property into a Multi-Point Stimulation method in which the nerve is stimulated at a number of different sites, and only the first few MUs recruited at each site are incorporated into the representative MU sample. While this method overcomes the probabilistic activation problem, the issue of adequate MU representation remains (cf. Section 2.3.1).

Since the VMA methods use voluntary muscle activation to recruit their MU samples, they are not affected by problems associated with probabilistic MU activation. However, they are not without their own problems. Specifically, the single-fiber triggering electrode may pick up temporally distributed potentials arising from fibers of the same MU or from different MUs, thus disturbing the triggering process (Brown et al., 1988). The triggering process may also break down under certain pathological conditions. Specifically, in diseased muscles, the single fiber electrode is
sensitive to variations in conduction delay, termed "jitter", or conduction blocks in terminal nerve twigs. Under these conditions, the single-fiber potential no longer serves as a stable trigger source.

2.3.4 Temporal Dispersion

In general, the individual MUAPs that summate to form the M-wave potential are not perfectly aligned with one another with respect to the time axis. That is, the peaks and nadirs of the MUAPs are temporally dispersed, resulting in what can been termed non-algebraic summation. This dispersion is due to the fact that the motor neurons do not all have identical conduction velocities. Rather, the conduction velocities are distributed over a range between 40 and 80 m/s (Cummins and Dorfman, 1981). An example illustrating the distance-dependent nature of this dispersion is shown in Figure 7.

Only one of the two MU estimation strategies takes temporal dispersion into account. In particular, in the ENS methods, the temporal alignment of the individual MUAPs with respect to the M-wave potential is preserved, and is taken into account when calculating the average MUAP amplitude. The VMA methods, on the other hand, do not provide any temporal information in terms of how the individual MUAPs should be aligned with respect to the M-wave potential. Therefore, in order to arrive at a MU estimate, it is assumed that the individual MUAPs summate in an algebraic manner. However, when the peaks of the MUAPs do not coincide with one another, the amplitude of the M-wave potential will be lower than the sum of the amplitudes of its constituent MUs. This results in an under-estimation of the number of MUs in the muscle.

Interestingly, Loof (1986) evaluated a similar problem which involved the temporal alignment of individual muscle fiber action potentials to their associated MUAP. Each muscle fiber action potential was aligned to the MUAP on the basis of the maximum correlation value between these
two signals. While this approach could readily be extended to our problem of aligning MUAPs to the M-Wave, there is no guarantee that the delay that yields the maximum correlation corresponds with the actual delay of the MUAP with respect to the M-wave. Specifically, let us assume that we are examining a muscle in which the shape of each MUAP is identical, but the latencies are not (i.e. each MU is associated with a different conduction velocity). Using Loof's correlation approach, the alignment of each MUAP with respect to the M-Wave potential would be identical. This is clearly incorrect. While this example oversimplifies the actual conditions, it nonetheless illustrates the shortcomings of this approach.
Figure 7: An example illustrating the effects of temporal dispersion. The top series of curves show the potentials that would be recorded from five MUs (each associated with a different conduction velocity) as a function of distance (d = 0, 10 and 50 cm for the left, center and right columns, respectively). The bottom curves were calculated by simply summing the five potentials immediately above them. It can be seen that as the distance increases, the amplitude of the summed response decreases.
2.4 The Error Associated with a Sampling Approach

2.4.1 Uncertainty Analysis

Little research has been directed towards evaluating the error that is associated with estimates obtained using the various methods. Specifically, the only reference to this in the literature comes from Jasechko (1987) who employed a technique called uncertainty analysis (Kline and McClintock, 1953). In uncertainty analysis, a range (termed the uncertainty interval) is established for a given calculation on the basis of the variation of the constituent parameters of the calculation. For the MU estimate calculation, these parameters are the M-Wave amplitude (M) and the amplitude of the mean MUAP contribution (m). The uncertainty in the estimate (designated as \( \delta_N \) where N is the MU estimate) can be expressed as follows:

\[
\delta_N = \sqrt{\left(\frac{\partial N}{\partial M} \delta_M\right)^2 + \left(\frac{\partial N}{\partial m} \delta_m\right)^2}
\]  

where \( \delta_M \) and \( \delta_m \) are the uncertainties of the M-Wave and mean MUAP amplitudes, respectively. \( \delta_M \) and \( \delta_m \) can be expressed in terms of their associated standard deviations, yielding the following

\[
s_N = \sqrt{\left(\frac{\partial N}{\partial M} s_M\right)^2 + \left(\frac{\partial N}{\partial m} s_m\right)^2}
\]  

where \( s_N \) is the uncertainty of the estimate (expressed in terms of a standard deviation), \( s_M \) is the standard deviation associated with the M-Wave assessment, and \( s_m \) is the standard deviation associated with the assessment of the mean MUAP contribution. Inserting equation [1] into the above yields the following

\[
s_N = \frac{1}{m} \sqrt{s_M^2 + N^2 s_m^2}
\]  

Of the two error sources, $s_m$ is much larger since we typically employ sample sizes that are only a fraction of the population size.

2.4.2 A Computer Simulation of the Sampling Procedure

Unfortunately, uncertainty analysis does not provide us with an idea of the error associated with given sample and population sizes. Therefore, we have evaluated this more general problem through the use of a computer model of the sampling problem (Slawnych, Laszlo, and Hershler, 1990a).

The modeling procedure consists of the following steps. Firstly, we specify the distribution of the MUAP amplitudes. We then specify the population size (i.e. the number of MUs in the muscle), and use an appropriate random number generator to assign an amplitude to each MUAP. The M-Wave amplitude is then calculated by summing the individual MUAP amplitudes. The number of MUs used to represent the MU population, termed the sample size, is specified and a random MU sample is obtained. The mean MUAP amplitude is then calculated and is divided into the M-Wave amplitude to yield the MU estimate. This sampling procedure is repeated 10,000 times, yielding 10,000 MU estimates. The mean and standard deviation of these estimates are calculated, with the latter serving as our index of estimation error.

Two different amplitude distributions were employed, shown in Figures 8a and 8b. These distributions were chosen since they qualitatively model the MUAP amplitude distributions observed under normal and neurogenic conditions, respectively. In particular, in normal muscles, the majority of the motor unit potentials have small amplitudes, although there are some potentials with larger amplitudes (Brown, 1984). In a muscle undergoing a neurogenic disease process, on the other hand, the amplitudes of the motor unit potentials shift to higher values, reflecting the reinnervation process that usually takes place.
Using these two distributions, we evaluated the estimation error obtained for a population range of 100 to 1000 MUs and 3 different sample sizes: 10, 15, and 20 MUs. The results are shown in Figure 8c. The curves with the highest errors for the control and neurogenic muscles correspond to a sample size of 10, with successive curves with lower errors corresponding to sample sizes of 15 and 20, respectively.

It is not surprising to see that the estimation error decreases with increased sample size and increases with increased population size. However, it is relevant to observe that the distribution modeling the normal population yields much higher estimation errors than those associated with the distribution modeling the neurogenic population. The basis for this observation is as follows. In the neurogenic case, the majority of the MUs have a large amplitude, and hence the MU sample will mostly consist of the large amplitude MUs. If a smaller amplitude MU is introduced to the sample, the average MU amplitude will decrease. However, the magnitude of this decrease will be smaller than the magnitude of the increase that we would see if a large amplitude MU is introduced to a sample consisting of small amplitude MUs. In addition, neurogenic disease processes are associated with motor unit loss, hence reducing the population size. This should also lower the estimation error associated with neurogenic muscles.

In Table 1, we tabulate the error values for three specific MU populations: 150, 300 and 900 MUs. These populations were chosen since they represent estimates typically obtained for the extensor digitorum brevis [EDB], the thenar muscles, and the biceps-brachialis, respectively. The error values indicate that it would be difficult to detect preliminary denervation in large muscles such as the biceps-brachialis.

It is important to note that the error values shown in Figure 8c and Table 1 are only estimates of the actual error values since the distribution functions for the MU amplitudes are not explicitly known.
Sampling error would not be a concern if all of the MUAPs were identical to one another. Unfortunately, there are several reasons why this is not the case. Firstly, there may be a large variation in the number of fibers comprising each MU. Secondly, the fibers from each MU occupy a unique sub-space of the muscle. Hence, more superficial MUs make a larger contribution to surface recorded potentials than more distant MUs. In an effort to eliminate this inequality, some investigators (Wee and Ashley, 1990) have proposed that the recording location be removed from a site directly overlying the muscle and placed more remotely. In such a recording arrangement, however, volume conducted activity from other muscles may corrupt the activity originating from the muscle of interest.

Table 1: Estimate Ranges for Various Muscles

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Estimate Range as a Function of Sample Size</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>$N_{\text{Sample}} = 10 \text{ MUs}$</td>
</tr>
<tr>
<td>EDB (N=150)</td>
<td>150±39</td>
</tr>
<tr>
<td>thenar (N=300)</td>
<td>300±80</td>
</tr>
<tr>
<td>biceps-brachialis (N=900)</td>
<td>900±245</td>
</tr>
</tbody>
</table>
Figure 8: a) Distribution modeling the normal motor unit population. b) Distribution modeling the neurogenic motor unit population. c) Estimation error (expressed in terms of a standard deviation) as a function of population and sample size.
2.5 Alternative Methods of Obtaining the Representative MU Sample

There exists a whole new class of techniques that have the future potential to be useful in obtaining MU estimates. These are the specialized EMG techniques that extract individual MUAPs from EMG signals recorded in response to low-to-moderate force voluntary contractions. Automatic Decomposition Electromyography [ADEMG] (McGill et al., 1985) is an example of such a technique. The procedure involves recording the muscle activity with a standard concentric needle electrode and, through a series of four signal processing steps, identifying constituent MUAPs. It may then be possible, by supra-maximally stimulating the nerve to the muscle, to obtain the maximal muscle potential. The MUE could then be calculated by dividing the mean MUAP amplitude into the amplitude of the maximal muscle potential.

Unfortunately, the majority of these decomposition techniques use an invasive recording protocol, and hence would only yield local MU densities as opposed to true MU estimates. McGill and colleagues investigated the feasibility of modifying their method to incorporate surface recordings, thus increasing the applicability of this type of an approach to MU estimation (McGill et al. 1987). However, research into surface recording based decomposition techniques is still in its infancy, and much work remains to be done before such an approach becomes clinically viable. It should also be noted that decomposition based techniques would not provide any temporal information regarding the alignment of the MUAPs with respect to the maximal potential.

2.6 Methods Based on Analyzing Nerve Fiber Action Potentials

All of the methods discussed so far in this chapter obtain the raw information on MU activity by recording, one way or another, the MUAP. It is logical to examine the question of whether the potentials generated by the neural component of the nerve/muscle system would be a better starting point for MU estimation. At first glance, it seems that by using nerve fiber action potentials [NFAPs], many of the problems of the ENS and VMA methods can be eliminated. In particular, nerve fibers are contained within a relatively small cross-section and the distance from
a surface recording site to the nerve is large compared to the inter-fiber distances. On this basis, one would be tempted to assume that the NFAP waveforms are identical to one another. This being the case, only a single NFAP would be required to arrive at the mean NFAP amplitude (that is, the sampling error is effectively eliminated). Unfortunately, the NFAP waveforms are not all identical. Rather, they vary with the conduction velocity of the nerve fiber which in turn varies with the diameter of the fiber. The exact nature of this relationship is unknown, however most investigators assume that NFAPs have identical shapes and only differ in terms of their amplitudes. Current estimates of this weighting factor range from a linear dependence to one in which the amplitude varies as the square of the velocity (Barker et al., 1975; Cummins et al., 1979).

An additional limitation stems from the fact the we are no longer able to distinguish the potentials generated by the alpha motor neurons from the remaining sensory and motor components. Specifically, nerves consist of both sensory and motor components. The motor component can be further sub-divided into alpha and gamma components. The gamma fibers innervate the muscle spindles which play a major role in tension regulation, while the alpha fibers innervate the muscle fibers that constitute the MUs. Therefore, the contribution of the alpha fibers to the maximally evoked nerve potential would somehow have to be estimated. Due to our limited knowledge concerning the relative proportions of different fiber types in the peripheral nerves, this estimation would be difficult. In addition, a fixed estimate of the relative proportion of alpha fibers would not take into account inter-subject differences in nerve fiber composition.

Finally, NFAPs are much smaller than MUAPs. In particular, the maximal potentials recorded from nerve fibers are on the order of 20 μV. Hence individual NFAPs have amplitudes in the nV range which is below the electromagnetic noise level of both the instrumentation and the environment. While environmental electromagnetic noise levels could be reduced by appropriate shielding, it would be difficult to reduce the instrumentation noise to this level.
3. MUESA - MOTOR UNIT ESTIMATION BASED ON STOCHASTIC ACTIVATION

3.1 Introduction

The development of MUESA began with a critical evaluation of the methods examined in Chapter 2. In general, all of these methods share the following steps:

1. obtaining the summated contribution of the entire MU pool (the M-Wave response),
2. obtaining the representative MU sample and using this sample to calculate the mean MU contribution to the M-Wave response, and
3. calculating the MU estimate by dividing the mean contribution into the full contribution.

Obtaining the M-Wave response is relatively straightforward, as the nerve is simply supramaximally stimulated. The actual calculation of the MU estimate is also straightforward. It is the second step where most of the difficulties have been encountered, and is where we have directed our efforts.

As we discussed in Chapter 2, there are two different ways in which we can obtain the representative MU sample: either using electrical stimulation or voluntary muscle contraction. We chose to adopt the former since

1) it incorporates temporal information into the calculation of the mean MUAP amplitude, and
2) it is non-invasive, making it clinically more attractive.

As stated in Section 2.3.3, the difficulty in obtaining the representative MU sample stems from the probabilistic manner in which MUs are activated in response to external electrical stimulation. While the majority of the ENS approaches view the probabilistic activation of multiple MUs as a problem which must be overcome, we have developed a method that capitalizes on this phenomenon. In particular, the threshold variability inherent in the system permits us to develop a method based on the delivery of constant-intensity stimulus trains. For this reason, we have
termed our method MUESA - Motor Unit Estimation based on the Stochastic Activation of motor neurons in response to external electrical stimulation.

3.2 Basic Description of MUESA

We begin with the acquisition of the M-Wave response which is obtained by supra-maximally stimulating the nerve innervating the muscle. We then subject the nerve to trains of constant-intensity stimuli, yielding a number of response sequences. The delivery of each stimulus train generally results in the probabilistic activation of a small number of MUs. While the idea of employing constant-intensity stimulus trains is not, in itself, a new concept (Milner-Brown and Brown, 1976; Kadrie and Brown, 1978, 1978a), our analysis of the resultant potential sequences is novel. We begin by determining the number of unique responses present in each sequence. The majority of the estimation methods employ the template matching approach to perform this classification. Instead, we use a clustering procedure since it yields classifications that are not influenced by the input order of the responses. The specification of the threshold classification level is also of critical importance to the classification procedure. While some investigators have employed arbitrary threshold values, our values are based on noise levels encountered during the course of the examination. The classified responses are then processed by a novel decomposition routine that also capitalizes on the probabilistic activation framework.

In the remainder of this Chapter, we show how stochastic, or probabilistic, MU activation can be exploited to obtain the representative MU sample.

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The terms "stochastic" and "probabilistic" are used throughout this thesis to describe the manner in which MUs are activated in response to external electrical stimulation.
3.3 Capitalizing on Probabilistic MU Activation

3.3.1 Basic Principles

There are three different ways that a motor neuron, and hence a MU, can respond to repetitive, constant-intensity stimulation:

1) it may always be activated,
2) it may be probabilistically activated, or
3) it may never be activated.

An example of this is illustrated in Figure 9. Specifically, if the stimulation intensity is 6.5 mA, motor neurons 1 and 2 will always be activated, motor neurons 3 and 4 will be probabilistically activated, and motor neuron 5 will never be activated.

![Figure 9: Classifying motor neurons on the basis of where the stimulus intensity intersects their associated activation curves.](image)

No information can be obtained from the group of MUs that are always activated since we generally do not know how many MUs comprise this group. Similarly, no information can be obtained from the group of MUs that are never activated since these MUs do not contribute to the recorded potentials. However, information can be obtained from the potentials produced by the
probabilistically activated MUs. In particular, let us examine the general case in which we are stimulating the nerve at an intensity that intersects the firing curves of n MUs. There are $2^n$ possible ways in which that this system can respond to a single stimulus pulse, representing different combinations of active and inactive MUs. For example, if $n=2$, the following four responses are possible:

- $R_1$: both MU$_1$ and MU$_2$ are not active,
- $R_2$: MU$_1$ is active and MU$_2$ is not active,
- $R_3$: MU$_1$ is not active and MU$_2$ is active, and
- $R_4$: both MU$_1$ and MU$_2$ are active.

In response to N stimuli, N stimulus-evoked muscle potentials are recorded. Collectively, these potentials are referred to as the *observed response sequence*. In general, this sequence will consist of a relatively small number of unique potentials, which are referred to as the *unique response set*. If the unique response set contains all $2^n$ possible responses, then it is termed a *full response set*. Otherwise, the response set is termed *incomplete*. An example illustrating the use of this terminology is shown in Figure 10.
Figure 10: a) A series of potentials recorded in response to constant-intensity stimulation. The numbers adjacent to the potentials refer to the order in which the potentials were observed. Collectively, all of the potentials are referred to as the response sequence. b) The unique potentials which comprise the response sequence shown in a). Collectively, these unique potentials are referred to as the unique response set. In this particular case, we have an incomplete response set since the number of unique potentials is not equal to an integer power of 2.
3.3.2 Analysis of the Case in which a Single MU is Undergoing Probabilistic Activation

There are two different ways that a single MU undergoing probabilistic activation can respond to a single stimulus pulse: either it is not activated or it is activated. We designate these two responses $R_1$ and $R_2$, respectively. There are $2^N$ different ways the system can respond when the nerve is stimulated $N$ times. Table 2 lists the eight different response sequences that are possible when $N=3$.

Table 2: The different ways a single MU undergoing probabilistic recruitment can respond to three stimulus pulses.

<table>
<thead>
<tr>
<th>Response Observed After Stimulation</th>
<th>1st Pulse</th>
<th>2nd Pulse</th>
<th>3rd Pulse</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$R_1$</td>
<td>$R_1$</td>
<td>$R_1$</td>
</tr>
<tr>
<td></td>
<td>$R_1$</td>
<td>$R_2$</td>
<td>$R_1$</td>
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<td>$R_2$</td>
<td>$R_2$</td>
<td>$R_1$</td>
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<tr>
<td></td>
<td>$R_2$</td>
<td>$R_2$</td>
<td>$R_2$</td>
</tr>
</tbody>
</table>

Looking at Table 2, we see that six out of the eight sequences contain at least one of each of the $R_1$ and $R_2$ responses, and hence they are termed full response sets. If any of these sequences are indeed observed, the MUAP associated with the probabilistic activation of the MU can be simply obtained by subtracting $R_1$ from $R_2$, as shown in Figure 11.
Figure 11: Example of a situation in which one MU is undergoing probabilistic activation in response to a stimulus train consisting of 40 stimulus pulses. a) The recorded response sequence. b) The unique response set, which in this case is also the full response set. c) The MUAP extracted from this sequence.
Table 2 also shows that there are two sequences which contain only one unique response. In this case, no information can be obtained, since only one unique potential is observed. Therefore, in order to be able derive any information from the system, it is essential that both the \( R_1 \) and \( R_2 \) responses are observed. Given that the MU is activated with probability \( P \), the probabilities of observing \( R_1 \) and \( R_2 \) in response to a single stimulus pulse are \( 1-P \) and \( P \), respectively. Correspondingly, in response to \( N \) stimuli, the probabilities of exclusively observing either \( R_1 \) or \( R_2 \) are \( (1-P)^N \) and \( P^N \), respectively. Hence, the probability of observing a sequence in which both \( R_1 \) and \( R_2 \) are present (designated as \( P_{\text{full}} \)) is given by the following

\[
P_{\text{full}} = 1 - (1-P)^N + P^N
\]

\( P_{\text{full}} \) is plotted in Figure 12 as a function of \( P \). As we can see, \( P_{\text{full}} \) is maximum when the activation probability for the MU is 0.5. Any deviation from this value reduces the probability of observing both responses. Not surprisingly, \( P_{\text{full}} \) increases as a function of the number of stimuli. This is better illustrated in Figure 13, where \( P_{\text{full}} \) is explicitly plotted as a function of the number of stimuli.
Figure 12: The probability of observing both responses as a function of the activation probability of the MU. The lowest curve corresponds to 5 stimuli, with successive curves corresponding to 10, 20, and 40 stimuli, respectively.

Figure 13: The probability of observing both responses as a function of the number of stimuli ("P" indicates the probability of activation for the MU).
3.3.3 Analysis of the Case in which Multiple MUs are Undergoing Probabilistic Activation

As we have shown, it is relatively straightforward to obtain the MUAP associated with the probabilistic activation of a single MU. Unfortunately, only the first few MUs can generally be activated independently of the remaining MUs. As the stimulus intensity is increased, multiple MUs tend to be probabilistically activated. Hence, repetitive stimulation may elicit a number of different responses, corresponding to different combinations of active and inactive MUs.

Ideally, we would like to observe each of the $2^n$ possible responses at least once within the sequence of $N$ observed responses. That is, we would like to have the full response set, since it is relatively straightforward to extract the constituent MUAPs in these cases. For example, when $n=2$, the two MUAPs can be obtained by subtracting the smallest response from both the second and third smallest responses. The general procedure to extract MUAPs when full response sets are observed is given in Appendix 4.

*Probability of Observing a Full Response Set*

Let us now examine the probability of observing the full response set as a function of $n$, $N$, and the MU activation probabilities. To simplify matters, let us assume that all $n$ MUs are activated with a probability of 0.5. Hence, each of the $2^n$ possible responses has an equal probability of being observed. We are then left with the more general problem of enumerating the number of different ways we can organize $N$ responses into $2^n$ MU categories such that each category contains at least one response. The solution to this problem is given by Jackson and Thoro (1990) as

$$N_{full} = (2^n)! \cdot S_{N,2^n}$$  \hspace{1cm} [10]$$

where $S_{N,2^n}$ is the Stirling number of the second kind and is defined as follows:
Since the number of possible response sequences is given by \( (2^n)^N \), the probability of observing the full response sequence is given by the following

\[
S_{N,2^n} = \frac{1}{(2^n)!} \sum_{j=0}^{2^n} (-1)^{2^n-j} \frac{(2^n)!}{j! (2^n - j)!} j^n
\]

[11]

\[
P_{\text{full}} = \frac{(2^n)! S_{N,2^n}}{(2^n)^N}
\]

[12]

\( P_{\text{full}} \) is plotted in Figure 14 as a function of both the number of stimuli and the number of MUs undergoing probabilistic activation. As we can see, as the number of probabilistically active MUs increases, larger numbers of stimuli are required to observe the full response set.
Figure 14: The probability of observing all of the possible responses as a function of the number of stimuli and the number of MUs undergoing probabilistic activation. In each case, it is assumed that the firing probability of each MU is 0.5. The number adjacent to each curve indicates the number of MUs undergoing probabilistic activation.

An alternative approach has to be employed to calculate $P_{\text{full}}$ in those cases in which the MU activation probabilities are not equal to 0.5. In particular, we employed a "brute-force" method in which we calculated the probability of observing every possible sequence in which each of the $2^n$ different responses was present at least once. The results for the case in which two MUs are undergoing probabilistic activation are shown in Figure 15.
Figure 15: The probability of observing all of the possible responses as a function of the number of stimuli and firing probability for the case of 2 MUs undergoing probabilistic activation.
3.3.4 Factors Limiting the Number of Stimuli that we can Employ

Given that a sufficient number of stimuli can be delivered, it is theoretically possible to observe the full response set for any number of MUs undergoing probabilistic activation. However, in practice, there are several factors that we have to consider. Firstly, we must insure that the nerve/muscle system remains stationary over the course of the stimulation period. That is, we do not want any new MUs becoming probabilistically activated during the course of the stimulus train. While we have found that the system remains stationary when relatively small numbers of stimuli are employed (on the order of 50), the same may not be true for larger numbers of stimuli. In particular, small changes in tissue temperature over the course of long stimulation periods could possibly alter the MU firing probabilities. Subject restlessness also comes into play. During the course of the delivery of the stimulus train, it is important the subject remain motionless as not to move the stimulator and hence perturb the stimulator/nerve interface. This may be difficult for some individuals if the stimulus trains have long durations. Finally, the general comfort of the subject must be considered. While the majority of subjects do not report the stimulation as being painful, they do nonetheless find it mildly discomforting. The discomfort level increases with both the stimulation frequency and the number of stimuli that are delivered.

As a result of the above considerations, we are limited in terms of the number of stimuli that can be delivered in each stimulus train. Hence, there may be instances in which full response sets are not observed. For example, the response sequence shown in Figure 10 contains only three unique responses and thus represents an incomplete response set. There are six different ways that we can interpret these responses in terms of our probabilistic framework, as given in Table 3. Interpretations 4, 5 and 6 are effectively the same as interpretations 1, 2 and 3, respectively, with only the labels assigned to the two MUs interchanged. Without any additional knowledge, it is not be possible for us to immediately determine which is the correct interpretation. The problem becomes even more complicated when there are more MUs undergoing probabilistic activation.
Therefore, we have developed methods to deal with incomplete response sets. These methods are discussed in the next section.

<table>
<thead>
<tr>
<th>Table 3: Interpretation of an incomplete response set</th>
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<tbody>
<tr>
<td>Interpretation</td>
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<tr>
<td>Number</td>
</tr>
<tr>
<td>--------</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
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<tr>
<td>3</td>
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<td>4</td>
</tr>
<tr>
<td>5</td>
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<tr>
<td>6</td>
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</tbody>
</table>

3.4 Analysis of Incomplete Response Sets

In general, there are two different ways that we can deal with incomplete response sets. We can either analyze the set "as is", or alternatively, we can attempt to acquire the missing response(s).

3.4.1 Acquiring the Missing Responses

There are two different ways in which we can attempt to acquire the missing responses. Firstly, instead of employing constant-intensity stimulus trains, we can introduce small, random perturbations to the stimulus intensities. However, we found that incorporating these perturbations into the stimulus trains generally did not increase the probability of obtaining all of the responses. Increasing the amplitude of these perturbations may increase this probability, but it may also result in the activation of new MUs that were previously inactive, hence complicating our task.

Secondly, we may utilize information obtained from other sequences elicited in response to stimulation at different intensities. However, there may be instances in which it is difficult
determine which of the responses from the additional sequence(s) represent the missing response(s).

3.4.2 Analyzing Each Set "As Is"

Evaluating the Amplitude Range (Method of Milner-Brown and Brown)

Let us consider the general case in which we have \( n \) MUs undergoing probabilistic activation. Designating the \( j^{th} \) MUAP as \( a_j(t) \), we have that the mean MUAP amplitude (\( m \)) is given by the following:

\[
m = \frac{\text{amplitude of } a_{\text{all}}(t)}{n}
\]

where

\[
a_{\text{all}}(t) = a_1(t) + a_2(t) + \ldots + a_n(t)
\]

When dealing with muscle potentials elicited in response to constant intensity stimulus trains, the individual MUAPs are not generally directly observed. Rather, we observe a sequence of responses representing different combinations of active and inactive MUs. If all \( 2^n \) possible responses are observed, then one of the responses will represent the case in which none of the MUs undergoing probabilistic activation are active. Similarly, another one of the responses will represent the case in which all of the MUs undergoing probabilistic activation are active. Designating these responses \( r_{\text{null}}(t) \) and \( r_{\text{all}}(t) \), respectively, we have that

\[
r_{\text{all}}(t) = r_{\text{null}}(t) + a_1(t) + a_2(t) + \ldots + a_n(t)
\]

Hence

\[
m = \frac{\text{amplitude of } (r_{\text{all}}(t) - r_{\text{null}}(t))}{n}
\]
If we do not have all $2^n$ responses, then we can estimate $m$ as follows:

$$m_{\text{est}} = \frac{\text{amplitude of } (r_{\text{max}(t)} - r_{\text{min}(t)})}{\lceil \log_2(N_{\text{unique}}) \rceil} \quad [17]$$

where $r_{\text{max}}(t)$ and $r_{\text{min}}(t)$ are the largest and smallest observed potentials, respectively, $N_{\text{unique}}$ is the number of unique responses in the observed response sequence, and $\lceil \log_2(N_{\text{unique}}) \rceil$ represents the ceiling of $\log_2(N_{\text{unique}})$ which is interpreted as the nearest integer of $\log_2(N_{\text{unique}})$ towards infinity. In simpler terms, $\lceil \log_2(N_{\text{unique}}) \rceil$ represents the minimum number of MUs that could generate the observed response sequence.

The calculation given in equation [17] was initially proposed by Milner-Brown and Brown (1976). In cases in which both $r_{\text{null}}(t)$ and $r_{\text{all}}(t)$ are observed, it yields the same solution as equation [16]. However, if either or both of these responses are missing, then we have that

$$r_{\text{max}}(t) - r_{\text{min}}(t) < r_{\text{all}}(t) - r_{\text{null}}(t) \quad [18]$$

in which case equation [17] will under-estimate of the mean MUAP amplitude, which in turn will result in the over-estimation of the number of MUs in the muscle.

---

For example, if $N_{\text{unique}} = 7$, then \( \log_2(N_{\text{unique}}) = 2.81 \) and hence $\lceil \log_2(N_{\text{unique}}) \rceil = 3$. 
**A Novel MUAP Extraction Method Based on Observation Rates**

In using constant-intensity stimulus trains, we are relying on the stochastic nature of motor neuron activation to activate a limited number of MUs. We can further capitalize on this stochastic activation framework to obtain MUAP(s) from incomplete response sets. Let us proceed by considering the case in which we have two MUs undergoing probabilistic activation. Table 4 outlines the four possible responses, along with their associated observation probabilities.

<table>
<thead>
<tr>
<th>Response</th>
<th>Status of MU&lt;sub&gt;1&lt;/sub&gt;</th>
<th>Status of MU&lt;sub&gt;2&lt;/sub&gt;</th>
<th>Probability of Observing the Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>R&lt;sub&gt;1&lt;/sub&gt;</td>
<td>inactive</td>
<td>inactive</td>
<td>((1-P_1)(1-P_2))</td>
</tr>
<tr>
<td>R&lt;sub&gt;2&lt;/sub&gt;</td>
<td>active</td>
<td>inactive</td>
<td>(P_1(1-P_2))</td>
</tr>
<tr>
<td>R&lt;sub&gt;3&lt;/sub&gt;</td>
<td>inactive</td>
<td>active</td>
<td>((1-P_1)P_2)</td>
</tr>
<tr>
<td>R&lt;sub&gt;4&lt;/sub&gt;</td>
<td>active</td>
<td>active</td>
<td>(P_1P_2)</td>
</tr>
</tbody>
</table>

These observation probabilities are shown in Figure 16 as a function of the two MU activation probabilities.
Figure 16: The probability of observing each of the four possible responses as a function of the activation probabilities of the two MUs.

We can map the two dimensional probability space in terms of which response has the greatest probability of being observed, termed the \textit{first most prevalent response}, or more simply, the \textit{most prevalent response}. Similarly, we can also map the second, third and fourth most prevalent responses. These maps are shown in Figure 17, along with their associated probabilities.
Figure 17: Mapping the probability space in terms of the probability of observing the four possible responses.
If any given response sequence contains only three unique responses, then we are most likely missing the 4th most prevalent (or, equivalently, the least prevalent) response. Given that this indeed is the case, there exists a method by which we can obtain both of the constituent MUAPs. Namely, the difference between the most prevalent and 2nd most prevalent responses represents a MUAP, as does the difference between the most prevalent and 3rd most prevalent responses. For example, consider the response sequence shown in Figure 18a. This sequence is comprised of four unique responses, as shown in Figure 18b. As we can see, response \( R_3 \) is associated with a low observation probability as it is only observed once. Therefore, let us assume that we do not observe this response. This leaves us with the remaining three responses, namely \( R_1, R_2 \) and \( R_4 \). Of these responses, \( R_2 \) is observed the most, and hence represents the most prevalent response. Evaluating the difference between 1) \( R_2 \) and \( R_1 \) and 2) \( R_4 \) and \( R_2 \) yields the MUAPs shown in Figure 18c. These are identical to the MUAPs that would be obtained if the original four responses were analyzed.

Figures 19a and 19b map the probability space in terms of which MUAP is obtained when the difference between the first and second most prevalent responses and also the first and third most prevalent responses are evaluated, respectively.
Figure 18: a) A sequence of responses recorded in response to a constant-intensity stimulus train. b) The unique responses which comprise the recorded sequence. c) The MUAPs obtained by subtracting the second and third most prevalent responses (namely R1 and R4) from the most prevalent response (R2).
Figure 19: Mapping the probability space in terms of which MUAP is recovered when a) the difference between the first and second most prevalent responses is evaluated and b) the difference between the first and third most prevalent responses is evaluated. The associated contour plots represent the summed probability of the two responses used to obtain the MUAP.

The use of this method is contingent upon one of the responses being clearly prevalent. However, there may be instances in which this is not the case. Specifically, when the activation probability of one of the MUs is 0.5, there will not be a prevalent response. For example, consider the case in which MU₁ is activated with probability $P_1 = 0.5$ and MU₂ is activated with probability $P_2 = 0.1$. The probability of observing each of the four possible responses are as follows: $P_{R_1} = 0.45$, $P_{R_2} = 0.45$, $P_{R_3} = 0.05$ and $P_{R_4} = 0.05$. Hence, both $R_1$ and $R_2$ are the most prevalent responses.
Fortunately, we can still extract a single MUAP by simply evaluating the difference between the two most prevalent responses.

Let us look at an example in which no clearly prevalent response is obtained. In particular, let us examine in greater detail the response sequence shown in Section 3.3. To simplify matters, this sequence is re-drawn in Figure 20a. This sequence consists of three unique responses, shown in Figure 20b. As we can see, responses $R_1$ and $R_2$ are observed almost an equal number of times. Hence, in this case, only one MUAP can be obtained, representing the difference between the $R_1$ and $R_2$ responses.

It should be noted that in the special case in which both MUs are activated with a probability of 0.5, all four responses have equal observation probabilities. However, this does not pose any problem since there is a high likelihood that the full response sequence will be observed.
Figure 20: a) A sequence of responses recorded in response to a constant-intensity stimulus train. b) The unique responses which comprise the recorded sequence. c) The MUAP obtained by evaluating the difference between the two most prevalent responses.
This type of analysis can be readily extended to the case in which there are three MUs undergoing probabilistic recruitment. In particular, a comprehensive evaluation of the three-dimensional probability space has shown that the difference between the two responses with the highest observation rates will correctly yield a MUAP (Appendix 5).

Since we are limited in terms of the number of stimuli we can deliver during the course of the stimulus train, it is difficult to extend this analysis for larger numbers of MUs. In particular, this analysis is based on comparing the relative observation rates of the various responses. Hence the number of stimuli should be significantly larger than the number of unique responses that are observed. For this reason, we have restricted our analysis to sequences in which there are three or fewer MUs undergoing probabilistic activation. Fortunately, this restriction is not as severe as it initially appears to be. In particular, we can exert some control over how many MUs are being probabilistically activated by minimizing the duration of the stimulus pulse, which effectively increases the threshold difference between different diameter nerve fibers (Gorman and Mortimer, 1983).
3.5 Discussion

The activation of motor neurons (and hence MUs) in response to external electrical stimulation is stochastic, or probabilistic, in nature, and has important implications for the ENS based methods. In particular, incremental increases in the recorded compound muscle potential may not necessarily correspond to the successive activation of individual MUs. Fortunately, we can capitalize on this probabilistic recruitment framework. Firstly, some level of control can be exercised over the system by using constant-intensity stimulus trains to probabilistically activate a limited number of MUs. Secondly, the observation rates of the unique responses can be used to decompose the observed response sequences into their constituent MUAP(s). In particular, while it is relatively straightforward to extract the constituent MUAPs from sequences in which all of the possible response combinations are observed, the decomposition of incomplete response sets is not a trivial problem. However, the difference between the two responses with the highest observation rates can be evaluated to yield a MUAP. The advantages of this observation-rate-based decomposition method over the Milner-Brown and Brown method are twofold. Firstly, as we have shown, there are instances in which the latter method will underestimate the mean MUAP amplitude. Secondly, the observation-rate-based method yields the actual MUAP waveforms, and hence the influence of temporal dispersion can be taken into account when calculating the mean MUAP amplitude. The disadvantage associated with the observation-rate-based method is that, unless one response is clearly prevalent, only a single MUAP can be extracted from each response sequence. It should also be noted that in using this method, we are assuming that the nerve/muscle system remains stationary during the course of the stimulation. While there is no way of guaranteeing the stationarity of the system over the long term, we can have reasonable expectations of stationarity over the observation period.
4. CLASSIFICATION OF THE OBSERVED RESPONSE SEQUENCES

Before we can extract the MUAPs from the observed response sequences, it is first necessary to divide each sequence into its constituent response classes, with each class representing a unique combination of active and/or inactive MUs. A difficulty arises in that the observed responses are usually associated with noise. That is we have

\[ x_i(t) = a_j(t) + e(t) \]  \[19\]

where \( x_i(t) \) is the \( i \)th response of the observed response sequence, \( a_j(t) \) is the \( j \)th response class and \( e(t) \) is the background noise. Because of this noise, it may not be immediately obvious to which class a particular response should be assigned. Therefore, a classification procedure must be employed. The literature in the field of classification is vast, and hence it would not practical for us to provide a comprehensive review of the myriad classification techniques. Instead, the purpose of this chapter is to provide a brief overview of the steps common to many classification procedures, and in doing so, specify the classification procedure that is optimal for our particular needs.

For the purpose of our analysis, we have broken down the classification procedure into the following four steps:

1. the extraction of features from the observed responses,
2. the selection of a distance measure,
3. the specification of the minimum distance between response classes, and
4. the assignment of the responses to the response classes.

These steps are discussed below.
4.1 Feature Extraction

Classification usually begins with the extraction of features from each response. Such extraction may utilize either time-domain or frequency domain features. Examples of the former include amplitude, area, latency and duration measures (Calvin, 1973; Camp and Pinsker, 1979; Gerber et al., 1984; Goodall and Horch, 1988; Yang and Shamma, 1988). Examples of the latter include both the coefficients of the Fourier series expansions and the power spectral densities of the responses (Bessou and Perl, 1969; Wheeler and Heetderks, 1979; Stashuk and deBruin, 1988). Some investigators have incorporated both time and frequency domain information into their classification schemes by looking at time-frequency maps obtained through the use of either short-time spectral methods or Wigner transformations (Babiloni et al., 1988). Unfortunately, none of these features guarantee an optimal classification.

Much attention has been directed towards the use of matched filters (Roberts and Hartline, 1975) since they are optimal for detecting a signal in noise (Dinning and Sanderson, 1981). Unfortunately, for single channel recordings, they are not optimal for distinguishing between event classes (Fukunaga, 1972; Wheeler and Heetderks, 1982). The scheme that does yield statistically optimal classifications for single channel recordings is template matching (Wheeler and Heetderks, 1982), and has been employed by us. In template matching, the data points themselves constitute the features, mapping the responses into a N dimensional hyperspace, where N is the number of points comprising each response.
4.2 Distance Measures

The next step after feature extraction is that of specifying the distances between the responses. Several different distance measures can be employed. The most common are the L1 (absolute value) and L2 (Euclidean) measures in which the distances between two responses $x_i(k)$ and $x_j(k)$ are given by:

\[ d_{ij} = \sum_{k=1}^{N} |x_i(k) - x_j(k)| \]  
[20]

and

\[ d_{ij} = \left( \sum_{k=1}^{N} [x_i(k) - x_j(k)]^2 \right)^{1/2} \]  
[21]

respectively, where $N$ is the number of constituent points of the responses. Of these two measures, L1 is usually employed since, with appropriate scaling, the calculations can be carried out in integer arithmetic. The L2 norm on the other hand requires the use of floating point arithmetic which is both more time and memory consuming than integer arithmetic. However, the L1 measure yields a sub-optimal classification which deteriorates with increased dimension (Dinning and Sanderson, 1981). This can be seen in the following example in which we have fixed the value of $N$ to 2. The decision boundaries for the L1 and L2 norms are a square and circle, respectively (Figure 21). For the L1 norm, the distance from the center of the acceptance region to a corner ($d_1$) is $\sqrt{2}$ times larger than the distance to the middle of one of the faces of the square ($d_2$). The L2 norm, on the other hand, maintains a constant distance between the center of the acceptance region and the boundary. For $N$ dimensions, this ratio becomes $\sqrt{N}$. Hence, all of our calculations were carried out using the L2 distance measure.
Figure 21: Evaluating the difference between the L1 and L2 distance norms. The decision boundary for the L1 norm is given by the square, whereas the decision boundary for the L2 norm is given by the circle.

4.3 Specification of the Minimum Distance Between Response Classes

For the most part, the specification of the confidence limits for different response classes has been based on empirical considerations rather than on strict mathematical formulations (cf. Popchev and Peneva, 1988). One of the few quantitative evaluations of this problem was carried out by Heetderks (1978), who derived minimum inter-response class distance values on the basis of two parameters: the desired correct classification rate (designated as $1-\alpha$) which is the probability of correctly attributing responses from the $i$th class to the $i$th class and the false alarm probability ($\beta_j$) which is the probability of incorrectly attributing responses from the $j$th class to the $i$th class. Specifically, we have that the minimum distance between any two response classes must satisfy the following:
\[ d_q \geq \sigma D \sqrt{N} \]  

where \( \sigma \) is the RMS noise level, \( D \) is the minimum separation value as specified by Heetderks (1978), and \( N \) is the dimension of the response space (i.e. the number of points used to represent each response). The value of \( D \) depends on the dimension of the response space, as shown in Figure 22 (the derivation of these values is quite involved and is given in Appendix 6).

**Figure 22:** Minimum required distance between response classes as a function of the confidence intervals and the dimension of the response space. The number pairs to the right of the curves indicate the \( 1-\alpha \) and \( \beta \) values, respectively.
4.4 Assigning the Responses to the Response Classes

We can break this problem into two cases. Firstly, there is the case in which we wish to assign the unclassified response \( x_i(t) \) to one of several pre-determined response classes \( a_i \). Secondly, there is the case in which we wish to classify the observed response sequence into their constituent classes without \textit{a priori} knowledge of these classes. It is this latter problem that we are faced with. There are many different techniques which can be used to achieve this type of classification, of which the classical template matching algorithm is one of the more popular. In template matching, a new response is compared to existing response templates and is assigned to the closest template if the response-to-template distance is below a certain threshold value. Otherwise, a new template is formed. Unfortunately, the classifications obtained using the template matching approach are sensitive to the input order of the responses to be classified. For example, consider the case in which we are classifying three responses on the basis of their amplitudes. Let us fix the amplitudes of these responses to 20 \( \mu \text{V} \), 32 \( \mu \text{V} \) and 40 \( \mu \text{V} \), and let us further fix the acceptance radius for the templates to 12 \( \mu \text{V} \). Table 5 lists the classifications on the basis of response order.

| Table 5: Examination of the influence of input order on response classification. |
|-----------------------------------------------|------------------|------------------|------------------|------------------|------------------|
| Input Response Order (Responses are specified on the basis of their amplitudes) | Constituent Responses(s) for each Template |
| First Response | Second Response | Third Response | Template 1 | Template 2 |
| 20 | 32 | 40 | 20,32 | 40 |
| 20 | 40 | 32 | 20 | 32,40 |
| 32 | 40 | 20 | 32,40 | 20 |
| 32 | 20 | 40 | 20,32 | 40 |
| 40 | 20 | 32 | 40 | 20,32 |
| 40 | 32 | 20 | 32,40 | 20 |
Of the 6 possibilities, 3 result in a classification in which the 20 μV and 32 μV responses form one template and the 40 μV response forms another, whereas the other 3 result in a classification in which the 20 μV response forms one template and the 32 μV and 40 μV responses form another.

Hence we must turn to alternative approaches, of which cluster analysis is particularly attractive. The objective of cluster analysis techniques is to sort a data set into categories such that the degree of association is high among members of the same category and low between members of different categories. One may be initially tempted to enumerate all of the possible combinations and choose the most appealing. Unfortunately, this quickly becomes intractable. In particular, the number of ways of sorting \( n \) observations into \( m \) groups is given by the Stirling number of the second kind

\[
S_{n,m} = \frac{1}{m!} \sum_{j=0}^{m} (-1)^{mj} \frac{m!}{(m-j)!} j^n
\]  

For the relatively small problem of sorting 25 observations into 5 categories, the number of possibilities is over \( 10^{16} \). The problem is further compounded by the fact that the number of groups is unknown, and hence the number of possibilities is a sum of Stirling numbers.

Cluster analysis techniques work by considering only a small number of the alternatives. A number of different approaches have been formulated (c.f. Hartigan, 1975; Spath, 1980). One of the more popular is termed K-Means clustering, and is based on minimizing the total within-group sum of squares error. This is achieved by first randomly selecting an initial partition and then modifying this partition by moving objects from one group to another if this movement reduces the sum of square error. Relocation continues until a local minimum of the sum of squares error is reached. Thus, the K-Means approach produces results that are locally optimal. In order to
achieve a more globally optimal solution, many initial cluster partitions are employed. However, the true globally optimum partition can only be guaranteed by evaluating all of the partitions, a practice we have shown to be impractical.

The other major clustering approach is termed hierarchical clustering in which objects are successively grouped on the basis of various distance criteria. For example, the nearest neighbor technique starts off by assigning each object to its own cluster. The clusters that are nearest to one another are then successively grouped until there is only one cluster. Hence each successive level contains one less cluster than the previous level. The actual number of clusters is then determined by evaluating the inter-cluster distances at each level.

All of our classifications were carried out using the nearest neighbor technique, which was chosen over the K-means method due to its computational efficiency.
5. IMPLEMENTATION

MUESA is implemented in terms of two modules: a data acquisition module and a data analysis module. These modules, which consist of C language routines, control all aspects of data acquisition and analysis. The modules are run on a 20 MHz 80386 based IBM compatible microcomputer equipped with a math coprocessor. The signals are recorded using a DISA model 1500 EMG system, in which the main components are a biopotential amplifier (model 15C01) and a nerve stimulator (model 15E25). The EMG system and computer are interfaced through a Lab Master 12-bit A/D converter.

5.1 Parameter Settings

The lower and upper frequency limits (3dB points) are set to 10 Hz and 1000 Hz, respectively, which covers the range of frequencies typically observed in surface recorded EMGs. The stimulus trains consist of constant-current pulses, 50 μsec in duration. The time between successive pulses is 500 msec. The signal recordings consist of 100 msec epochs, each represented by 250 data points. This corresponds to a sampling frequency of 2.5 kHz, which satisfies the Nyquist minimum sampling frequency criterion. The delivery of the stimulus pulse occurs at a time midway in the 100 msec recording epoch, such that both pre-stimulus and post-evoked potential segments are included with the stimulus-evoked potential (Figure 23). These segments are used to establish the noise level, and also provide a "window" from which to observe background muscle activity.
Figure 23: A typical response epoch which shows the pre-stimulus segment, the evoked potential segment and the post-evoked potential segment. The pre-stimulus and post-evoked potential segments are used to establish the noise level and to observe background muscle activity.

5.2 Data Acquisition Protocol

The data acquisition is carried out in three stages.

Stage 1: M-Wave Acquisition

Two maximal muscle responses are obtained by supra-maximally stimulating the nerve to the muscle. Ideally, we would like to record at least 10 M-Waves to establish the stationarity of the system. However, due to the high stimulus intensity levels associated with M-Wave acquisition, this is not feasible since it may reduce patient compliance.

Stage 2: Background Noise Acquisition

Four response epochs are then recorded with the stimulator turned off. These epochs serve to evaluate the background noise levels.
Stage 3: Sequence Acquisition

The threshold stimulation level at which responses are first observed is determined. The nerve is repetitively stimulated 50 times at this stimulus level, and the associated response sequence is recorded. This is then repeated using a number of different stimulus settings. The highest stimulus intensity used for each session typically elicited responses on the order of 15 to 20% of the M-Wave response.

Skin temperature is monitored throughout the evaluation, and if significant changes are observed, steps are taken to alleviate these changes. The sessions typically require 15 minutes to complete.

5.3 Data Analysis

At the completion of the data collection phase, data analysis begins and proceeds as follows.

Step 1: M-Wave Analysis

The amplitude and areas of the M-Wave responses are calculated. The M-Waves are also used to establish the time window with which to analyze the remaining compound responses.

Step 2: Noise Analysis

Noise statistics are then obtained, and if 60 Hz. activity is detected, a noise removal procedure is initiated. Specifically, a template of the 60 Hz noise is first constructed using the pre- and post-evoked potential segments. This template is then subtracted from the evoked potential segment. We found that this procedure yielded superior performance to that of a digital notch filter due to the "ringing" associated with the impulse response of the filter.
Step 3: Analysis of the Response Sequences

The series of potentials elicited at each level are analyzed as follows:

Pre-Analysis

The first ten responses in the sequence are discarded since they may be associated with transient changes (c.f. Section 5.4.4). The pre- and post-evoked potential segments of the remaining responses are examined for the presence of voluntary muscle activity. If activity is found in these segments, the response is discarded. This reduces the possibility of analyzing an evoked potential segment that may be corrupted with this background muscle activity. Otherwise the segments are used to establish the background RMS noise level.

Classification

The remaining responses are reduced to their unique response set using the nearest neighbor clustering technique discussed in Section 4.4. In performing this classification, the threshold levels are set such that the correct classification and false alarm rates are 95% and 5%, respectively (c.f. Section 4.3).

Decomposition

The constituent MU(s) are extracted using the observation-rate-based decomposition protocol described in Section 3.4.

Step 4: Data Evaluation

Once all of the sequences have been analyzed, the extracted MUAPs are used to generate the mean MUAP. The amplitude and area of this response are then calculated and divided into the amplitude and area of the M-Wave response, yielding the amplitude and area based estimates, respectively.
Figure 24 shows an example of the various stages of the analysis procedure. A more detailed description of both the acquisition and analysis procedures is given in Appendix 7.

Figure 24: An example illustrating the various steps employed to analyze the response sequences. a) The original sequence. b) The reduced sequence in which response epochs associated with excessive noise or spurious potentials are eliminated. c) The unique response set. d) The extracted MUs.
5.4 Special Considerations

5.4.1 Variability in the Observed Responses

Ideally, we would like to extract MUAPs from response sequences that are recruited over a full range of stimulus intensities such that the MU sample is representative of the entire MU pool. Unfortunately, this is not possible as individual MUAPs are associated with random amplitude and shape fluctuations (Milner-Brown and Brown, 1976; Guiheneuc, 1989; Haas and Meyer, 1989). As the number of active MUs increases, the magnitude of the net fluctuation correspondingly increases. Hence, the sensitivity of the method (in terms of detecting small amplitude MUAPs) decreases as the size of the evoked response increases. In general, the response fluctuation that we observe using a surface recording protocol is less than that observed when an invasive protocol is employed due to the averaging properties associated with surface electrodes. Nevertheless, some degree of fluctuation is present. Hence, our analysis was restricted to the lower end of the stimulus spectrum such that the largest responses that were analyzed were typically on the order of 15 to 20 percent of the M-Wave value.

5.4.2 Constant-Current Vs. Constant-Voltage Stimulation

In order for our approach to work, it is critical that the distribution and intensity of the electrical current within the tissue remain constant for a given level of external stimulation. For this reason, we have chosen constant-current over constant-voltage stimulation since the former is not sensitive to changes in the impedance of the electrode/skin interface.

5.4.3 Selection of Sampling Frequency

The sampling frequency that we employ is generally lower than that employed by other investigators. However, it is more than adequate since surface recordings do not have much signal power at frequencies above 700 Hz. (Kwatny, 1970; Monster et al., 1980). This was confirmed by examining the power spectral densities of signals that we typically record. Figure 25 shows an example in which two signals and their associated spectra are presented. As we can
see, there is very little signal power above 700 Hz. Our choice of sampling frequency was
governed by data storage considerations. In particular, a typical session in which 10 different
response sequences are acquired generates 256,000 bytes of data. Increasing the sampling
frequency would significantly increase this value.

![Figure 25](image)

**Figure 25:** Evaluating the power spectra associated with typical surface-recorded evoked
potentials. a) shows the compound potentials and b) shows their associated
spectra.

### 5.4.4 Selection of the Stimulation Frequency

The stimulation frequency plays a major role in determining the shape of the recorded potential.
In particular, Kadrie and Brown (1978) found that there was a progressive increase in the M-wave
amplitude with repetitive stimulation, and its magnitude increased with increased stimulation
frequency. We conducted a similar study in which we repeatedly stimulated the median nerve at
supra-maximal intensity levels at a number of different stimulation frequencies. At frequencies
above 1 Hz., five trains of 40 stimuli each were delivered, with a two minute interval between successive trains. Three trains were delivered at 1 Hz. The amplitudes and areas of the resultant responses were evaluated and are shown in Figures 26 and 27, respectively.

Looking at Figure 26, we see that three features stand out. Firstly, there is a progressive increase in the recorded amplitude as a function of the stimulus pulse number for any given stimulus train. Hicks and McComas (1988) found that this increase is associated with the progressive hyperpolarization of the individual muscle fibers which in turn is due to the electrogenic nature of the Na+K+ pump.

Secondly, we see that there is a small difference in the amplitude values between different stimulus trains. Specifically, we found that successive trains produced responses with higher amplitudes. This may represent some sort of cumulative hyperpolarization phenomenon.

Lastly, the signals recorded at stimulation frequencies of 6 Hz. and above tend to exhibit some additional random variability. This may represent movement artifact since these higher stimulation frequencies were generally associated with some degree of discomfort.

With the exception of the initial decrease as a function of the stimulus pulse number, the results obtained using the area values are very similar to the amplitude-based results (Figure 27). The initial decrease that we observe reflects a concomitant decrease in the peak-to-peak duration which may be due to an increase in the synchronization of the firing times of the constituent muscle fibers for each MU (Desmedt 1973; Kadrie and Brown, 1978).

In light of the observed amplitude and area variation, we restricted our stimulation frequency to 2 Hz. In addition, the first 10 potentials from each response sequence were discarded to eliminate the influence of early transient changes. While it may be possible to completely eliminate these
changes by further reducing the stimulation frequency, such a step would greatly increase data acquisition time.

It should be noted that there may be certain instances in which the system is not stationary even at the low stimulation frequencies. For example, in Amyotrophic Lateral Sclerosis [ALS], repetitive electrical stimulation results in a progressive decay in the amplitude of the compound potential (and hence the constituent MUs). Hence, electrical stimulation based estimation techniques would not be appropriate for the analysis of these conditions.

5.4.5 Number of Pulses per Stimulus Train
Each stimulus train consisted of 50 stimulus pulses. This was chosen in the interest of both minimizing the discomfort associated with the stimulation procedure, and also facilitating the stationarity of the nerve/muscle system.

5.4.6 Selection of the Pulse Duration
The duration of the stimulus pulse was set at 50 μs to maximize the threshold differences among different diameter nerve fibers (c.f. Section 3.4).

5.4.7 Computational Considerations
While the calculations required to calculate the actual estimates are not difficult, the preceding classification procedure is computationally intensive. In particular, if a K-Means clustering algorithm is employed with 100 different partitions being examined for each level, it takes on the order of 50 minutes to analyze the results obtained from a single muscle. Fortunately, this time can be dramatically reduced to the order of 15 minutes by employing the nearest neighbor clustering algorithm discussed in Section 4.4.
Figure 26: Influence of stimulation frequency on the M-Wave amplitude.
Figure 27: Influence of stimulation frequency on the M-Wave area.
6. EXPERIMENTAL RESULTS

In this chapter, we present the MU estimates obtained using MUESA, and compare them with estimates obtained using other methods. Two groups of subjects were employed for this investigation:

1) those with no reported neuromuscular deficits (designated as the control group), and
2) poliomyelitis subjects.

The latter group was used to evaluate the ability of our method to detect reduced MU numbers associated with neurogenic disease processes. Specifically, acute poliomyelitis is a viral disease which attacks the anterior horn cells in the spinal cord and the brain. Paralysis occurs if the motor neurons are destroyed. There is evidence suggesting that poliomyelitis subjects can be categorized into two different groups: those in which progressive muscle wasting and weakness are observed 20 to 30 years after the initial onset of the disease, and those in which no new deterioration is observed. The former class of symptoms has been termed post-polio syndrome (Nelson, 1990). For the purposes of this analysis, these two groups were not distinguished from one another.

6.1 Methods
6.1.1 Subjects

The control group consisted of 13 males and 15 females between the ages of 16 and 57. All of these subjects were in good general health and had no previous history of neuromuscular disease. The poliomyelitis group consisted of 7 males and 18 females between the ages of 38 and 75. All of the subjects in this group contracted the disease during their childhood. The diagnosis of poliomyelitis was confirmed by evaluating patient records and by performing both a physical examination and a standard electrodiagnostic examination. (Appendix 2 discusses the changes that occur to MUAPs during the course of neurogenic disease processes.) Informed consent was obtained in all cases prior to examination.
6.1.2 Muscles

Two different muscles were evaluated, the extensor digitorum brevis [EDB] and the thenar muscle group. The EDB is a muscle located on the dorsum of the foot and is responsible for the dorsiflexion of the toes. Its relatively superficial position facilitates surface recording, and the peroneal nerve is easily localized for surface stimulation. The thenar muscle group consists of four muscles: the abductor pollicis brevis, the opponens pollicis, the flexor pollicis brevis and the adductor pollicis. The first three muscles are covered by the thenar fascia which enclose them in their own compartment. The adductor pollicus is not enclosed by this compartment. The abductor pollicis brevis is the most superficial of the three muscles in the thenar compartment. These muscles are responsible for the abduction, flexion and rotation of the thumb. They are innervated by the median nerve which, like the peroneal nerve, is also readily localized for surface stimulation. In some subjects, the flexor pollicis brevis may be partially or entirely innervated by the ulnar nerve (Rowntree, 1949). Hence, the estimates that we derive for this muscle refer to the median innervated components. It is important to note that due to the random nature of the polio disease process, the EDB and thenar muscles were not necessarily affected in all of the polio subjects.

6.1.3 Electrode Placement

The EDB was examined with the subject lying in the supine position. The signals were recorded using 2.5 cm. x 0.5 cm. silver foil surface electrodes coated with a conductive paste and fixed in place with insulating tape. The stigmatic (active) recording electrode was positioned over the center of the muscle bulk such that it was aligned normal to the muscle axis, while the reference electrode was positioned just below the first digit. The ground electrode was positioned between the stigmatic and stimulating electrodes. The peroneal nerve was stimulated at the level of the ankle via a pair of gauze-padded, saline-soaked silver electrodes held in position with an elasticized strap.
The thenar muscles were examined in a similar fashion. The stigmatic electrode was positioned over the abductor pollicus brevis, with the reference electrode positioned at the tip of the first digit. In this case, the median nerve was stimulated at the level of the wrist.

6.1.4 Data Acquisition and Analysis

The signals were acquired and analyzed using the protocol described in Chapter 5. A number of subjects also underwent an examination in which the muscle activity elicited at full force voluntary contractions was recorded with a concentric needle electrode and analyzed in terms of the standard turns-amplitude assessment (Rose and Willison, 1967).

6.2 Results

Table 6 and Figure 28 present the estimates obtained from the control and polio subjects. In deriving these estimates, two different measures were employed, namely the response amplitudes and areas. While only amplitude-based estimates are generally calculated by the majority of investigators in this field, some investigators have advocated the use of area over amplitude measures (Ballantyne and Hansen, 1975). However, we found no significant difference in the estimates based on these two different measures.

| Table 6: Comparison of control and polio group estimates. |
|----------------|-----------------------------|-----------------------------|
| **Muscle**    | **Amplitude Based Estimates** | **Area Based Estimates**    |
|               | Control (N=23) | Polio (N=42) | Level | Control (N=23) | Polio (N=42) | Level |
| EDB           | 77±28          | 33±32         | p<0.01| 84±37          | 35±34         | p<0.01 |
| thenar        | 97±43          | 52±48         | p<0.01| 107±55         | 55±50         | p<0.01 |

4Level refers to the significance level calculated using the nonparametric Kolmogorov-Smirnov test.
5N indicates the number of subjects.
Figure 28: MU estimates obtained from the control and poliomyelitis groups.
The results indicate the there is a genuine difference in the number of MUs between the two subject groups. Specifically, as expected, lower estimates were found for the poliomyelitis group than for the control group. In addition, the MUAPs recorded from the poliomyelitis group were generally larger in amplitude and longer in duration than those recorded from the control group, which is what we would expect to see in a neurogenic disease process (c.f. Appendix 2).

In all cases, the difference between the control and polio group estimates is statistically significant (unless stated otherwise, the significance levels given in all of the tables were calculated using Student's t test). However, age differences between the two groups may at least partially account for the reduced estimates associated with the polio subjects. The age statistics for the two groups are given in Table 7.

| Table 7: Age statistics for the control and polio groups. |
|-------------------|-------------|-------------|-------------|
| Muscle | Subject Age | Control | Polio | Level |
| EDB | 33.2±12.5 | 52.2±9.7 | p<0.01 |
| thenar | 32.8±11.9 | 51.8±10.0 | p<0.01 |

As we can see, the polio subjects are significantly older than the control subjects. Several investigators have found reduced estimates associated with patients over the age of 60 (Brown, 1972; Campbell et al., 1973; McComas, 1977). We examined the correlation between age and the number of MUs and found no significant correlation between these variables for control subjects (Table 8). This is to be expected since all of the control subjects were less than 60 years of age. In addition, there was no significant correlation between age and the thenar estimates obtained from the polio group. However, we found that the EDB estimates were negatively correlated with age for this group. Hence some of the reduced estimates obtained from older polio subjects may be associated with the normal aging process. There is some histological
(Jennekens et al., 1972) and electrophysiological (Roselle and Stevens, 1973) evidence that suggests that denervation and reinnervation normally occurs in this muscle. Thus, the reduced estimates may also represent a normal "wear and tear" process for this muscle.

In order to eliminate the influence of age, we re-evaluated both groups by limiting our analysis to subjects less than the age of 50⁶. The results are presented in Table 9. Again, the two groups are statistically different on the basis of the MU estimates.

<table>
<thead>
<tr>
<th>Table 8: Examining the correlation between age and the motor unit estimates.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Muscle</strong></td>
</tr>
<tr>
<td><strong>Number of MUs</strong></td>
</tr>
<tr>
<td><strong>Control</strong></td>
</tr>
<tr>
<td>EDB</td>
</tr>
<tr>
<td>(p&lt;0.66)</td>
</tr>
<tr>
<td>thenar</td>
</tr>
<tr>
<td>(p&lt;0.34)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 9: Estimates obtained from subjects under the age of 50.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Muscle</strong></td>
</tr>
<tr>
<td><strong>Control</strong></td>
</tr>
<tr>
<td>EDB</td>
</tr>
<tr>
<td>(N=21)</td>
</tr>
<tr>
<td>thenar</td>
</tr>
<tr>
<td>(N=26)</td>
</tr>
</tbody>
</table>

⁶Although 60 years of age is the limit employed by other investigators, we found that this resulted in only a handful of subjects being excluded from the analysis. For this reason, we reduced our age limit to 50 years.

⁷The correlations were calculated using the Pearson correlation coefficient.
Looking at Figure 28, we see that several of the estimates obtained from the polio subjects fall within the control range. If we specify the minimum observed control estimate as the lower limit for normality, then 33 EDB and 25 thenar muscles would be classified as neurogenic on the basis of the standard amplitude-based estimates (the corresponding numbers for the area based estimates are 30 and 24, respectively).\(^8\) The remaining estimates are well within the control range, which reinforces our statement that not all of the muscles were necessarily affected by the disease process.

6.2.1 Comparison with the Turns-Amplitude Measure

As stated, a number of subjects also underwent concentric needle examinations, yielding a turns-amplitude assessment. Table 10 compares this assessment with that obtained using the MU estimates. More muscles were classified as neurogenic on the basis of the MU estimates than on the basis of the turns-amplitude assessment. Similar results were found by McComas and Sica (1978).

<table>
<thead>
<tr>
<th>Muscle Examined</th>
<th>Number of Muscles Classified as Neurogenic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MU Estimate Based Assessment</td>
</tr>
<tr>
<td>EDB (N=12)</td>
<td>9</td>
</tr>
<tr>
<td>thenar (N=9)</td>
<td>7</td>
</tr>
</tbody>
</table>

\(^8\)We chose to employ this limit as opposed to one based on the estimate statistics (e.g. the two standard deviation level) because of the non-Gaussian nature of the estimate distribution. A similar approach was used by Sica and McComas (1978).
6.2.2 Comparison with Control Estimates Obtained by Other Investigators

Table 11 compares our control estimates with those obtained by investigators employing the increment-counting technique and its derivatives.

<table>
<thead>
<tr>
<th>Investigators</th>
<th>Motor Unit Estimates</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>EDB</td>
<td>thenar</td>
</tr>
<tr>
<td>McComas et al. (1978)</td>
<td></td>
<td>212±67</td>
<td>343±97</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(N=112)</td>
<td>(N=115)</td>
</tr>
<tr>
<td>Brandstater et al. (1989)</td>
<td></td>
<td>135±76</td>
<td>267±121</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(N=20)</td>
<td>(N=20)</td>
</tr>
<tr>
<td>DeBruin et al. (1989)</td>
<td></td>
<td>248±75</td>
<td>453±257</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(N=23)</td>
<td>(N=22)</td>
</tr>
<tr>
<td>Galea et al. (1991)</td>
<td></td>
<td>131±45</td>
<td>228±93</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(N=30)</td>
<td>(N=33)</td>
</tr>
<tr>
<td>Slawnych et al. (1991)</td>
<td></td>
<td>87±37</td>
<td>107±37</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(N=23)</td>
<td>(N=29)</td>
</tr>
</tbody>
</table>

While the estimates obtained by the different investigators vary considerably, there is general agreement in that the thenar estimates are consistently higher than the EDB estimates. However, in terms of absolute values, our estimates are significantly lower than those obtained by the other investigators. There are three factors that may account for this difference. Firstly, our method directly deals with the problems associated with the stochastic nature of motor unit recruitment. In particular, as discussed in Chapter 2, if there are n MUs that are undergoing intermittent recruitment, we can expect to see up to $2^n$ different responses generated by different combinations of these MUs. In using the increment-counting technique, the difference between successive traces would be interpreted to represent the activation of new MUs, which would in turn result in the over-estimation of the number of MUs in the muscle.9

9As stated in Chapter 2, some of the newer methods based on the original increment-counting technique now employ what have been termed "alternation" detection algorithms to deal with the probabilistic recruitment issue.
Secondly, in obtaining our MU samples, we stimulated the nerve over a relatively large range of stimulus intensities, such that the amplitudes of the evoked compound potentials reached up to 20% of the M-Wave amplitude. Investigators employing other ENS based methods seldom examine compound potentials larger than 10% of the M-Wave amplitude. We found that the MUAPs that we extracted from the sequences recorded in response to the higher stimulation intensities generally had larger amplitudes than those extracted from the sequences observed at lower stimulation intensities. In particular, the amplitudes of the largest control MUAPs were 3.7±1.7 and 2.9±1.6 percent of the M-Wave value for the EDB and thenar muscles, respectively. In some cases, amplitudes as high as eight percent of the M-Wave value were obtained.

Lastly, in analyzing the response sequences, strict threshold levels were employed to determine the number of unique potentials in each response sequence. In particular, the classification thresholds were set such that the probabilities of correctly identifying responses from a given class to its appropriate class and of misclassifying responses from other classes were 0.95 and 0.05, respectively.

Over the course of the last several years, a number of new techniques have been used to estimate the number of MUs in the EDB and thenar muscles. These estimates, which are given in Table 12, are generally significantly lower than those obtained using the increment-counting technique.
Table 12: Recent estimates obtained using alternative methods.

<table>
<thead>
<tr>
<th>Investigators</th>
<th>Approach</th>
<th>Motor Unit Estimates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>EDB</td>
</tr>
<tr>
<td>Daube (1988)</td>
<td>&quot;Quantal&quot; Approach</td>
<td>185±32</td>
</tr>
<tr>
<td>Barkhaus et al. (1990)</td>
<td>Spike-Triggered Averaging Based Approach (EMG based)</td>
<td>48±10</td>
</tr>
<tr>
<td>Stein and Yang (1990)</td>
<td>Micro-Simulation Based Approach (EMG based)</td>
<td>135±27 (10)</td>
</tr>
<tr>
<td>Stein and Yang (1990)</td>
<td>Spike-Triggered Averaging Based Approach (EMG based)</td>
<td>122±38 (10)</td>
</tr>
<tr>
<td>Slawnych et al. (1991)</td>
<td>MUESA</td>
<td>87±37 (N=23)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>107±55 (N=29)</td>
</tr>
</tbody>
</table>

6.2.3 Reproducibility

Reproducibility was examined by evaluating a number of control subjects on three different occasions. Specifically, 6 EDB and 6 thenar muscles were examined, with several days separating each successive evaluation. The estimate statistics, presented in Table 13, indicate the extent of inter-session variability associated with the estimates. This variability can also be expressed in terms of a coefficient of variation in which each standard deviation is normalized by its respective mean. The mean coefficients of variation for the EDB and thenar muscles are 16.1 and 12.3, respectively, for the amplitude based estimates. The corresponding numbers for the area based estimates are 14.2 and 12.5, respectively. These numbers are comparable to those obtained by other investigators (c.f. Galea et al., 1991). As it stands, the method is not completely automated as the operator must select the intensities at which the nerve is stimulated. However, MUESA can readily be made fully automated, thus eliminating any subjective biases associated with the acquisition of the MU samples.
Table 13: Reproducibility assessment.

<table>
<thead>
<tr>
<th>Subject</th>
<th>EDB Estimates</th>
<th>Thenar Estimates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amplitude</td>
<td>Area</td>
</tr>
<tr>
<td></td>
<td>Based</td>
<td>Based</td>
</tr>
<tr>
<td>1</td>
<td>60±9</td>
<td>62±10</td>
</tr>
<tr>
<td>2</td>
<td>60±8</td>
<td>61±6</td>
</tr>
<tr>
<td>3</td>
<td>54±11</td>
<td>56±9</td>
</tr>
<tr>
<td>4</td>
<td>52±9</td>
<td>52±8</td>
</tr>
<tr>
<td>5</td>
<td>70±8</td>
<td>70±8</td>
</tr>
<tr>
<td>6</td>
<td>53±10</td>
<td>54±9</td>
</tr>
</tbody>
</table>

6.3 Discussion

The purpose of this experimental study was to evaluate MUESA - the new MU estimation method that we have developed. This evaluation was performed by obtaining estimates from both normal and poliomyelitis subjects. While our control estimates are similar to those obtained by other investigators employing the spike-triggered averaging based technique, they are significantly lower than those obtained by other investigators employing the increment counting technique. However, more emphasis should be placed on the relative estimates between control and disease groups than on the absolute number of MUs (c.f. Galea et al., 1991). That is, it is the relationship between the estimates obtained from control subjects and those with neuromuscular disorders that is of fundamental importance. In this context, we found that the MU estimates obtained from the polio group were significantly lower than those from the control group. In addition, emphasis should also be placed on the reproducibility of the results. The coefficients of variation that we obtained are well within the bounds observed by other investigators. As our modeling studies point out, reproducibility could be even further improved by increasing the sample size. This translates to increasing the number of stimulus trains that are delivered to the subject (all of the present results were obtained by employing a maximum of ten different stimulus trains for each subject). The use of additional stimulation levels would be particularly
useful in the examination of muscles in which the disease process is in its early stages, and also in following the progression of the disease.

The sizes of our MU samples are generally lower than that employed by other investigators. In particular, the sample sizes for the EDB and thenar muscles were 7.1±3.2 and 6.8±2.8, respectively. Again, increasing the number of stimulus trains would result in an increase in the sample size. It should be noted that we do not a priori know the size of the MU sample since the data is not analyzed until after all of the data has been acquired. The more powerful computer systems that are now available would allow one to analyze each response sequence immediately after it is recorded, hence alleviating this problem. Interestingly, there was never any real problem finding stimulus intensity levels which led to the probabilistic activation of one or more MUs, and hence elicited responses of varying amplitude. In particular, one may initially expect a reduction in the amount of variation observed in the signals recorded from the poliomyelitis individuals due to decreased MU numbers. However, this was not the case, and varying response sequences could always be elicited, even in the most severe cases.

Occasionally, repetitive stimulation yielded sequences which contained a continuum of responses in which there did not appear to be any discrete response classes. These sequences could either be the product of a large number MUs undergoing intermittent activation or a relatively small number of MUs which exhibit a large range of shape fluctuations. Further investigation of this issue is warranted.

While our examination was limited to the EDB and thenar muscles, MUESA could readily be extended to more proximal muscles such as the biceps-brachii. The assessment of these proximal muscles is particularly important since some diseases predominantly affect these muscles.
7. ALTERNATIVE ESTIMATION METHODS

In this chapter, we explore alternative methods for estimating the number of MUs in a muscle. The impetus behind the development of these methods is the elimination of the sampling error associated with both the current methods discussed in Chapter 2 and also MUESA. Specifically, the proposed uses of MU estimates are:

1) to objectively monitor the natural time course of disease processes,
2) to evaluate different therapies in a quantifiable manner, and
3) to facilitate the early detection of muscle denervation which is manifested by a decreased number of MUs.

Of these features, the early detection of muscle denervation has received the most attention (Brown and Feasby, 1974; Panayiotopoulos and Scarpalezos, 1975, Hansen and Ballantine, 1977; McComas and Sica, 1978). However, early detection is most likely not possible given the inherent sampling error associated with the MU estimates. One obvious way of eliminating this error would be to extend the sampling procedure to include all of the constituent MUs. However, this is not a viable approach since it becomes progressively more difficult to detect the activation of successive MUs (c.f. Sections 2.3.3 and 5.4.1). Therefore, we have developed three new estimation methods that move away from a sampling-based procedure.

7.1 A Method Based on Evaluating the System Response Function

7.1.1 Introduction

In most ENS based estimation strategies, the nerve is stimulated in proximity to the recording site, with the distance between the stimulation and the recording sites being on the order of 6 to 8 cm. Over these relatively short distances, the velocity distribution profile of the motor neurons does not play a major role in determining the shape of the maximal muscle potential. However, as the distance is increased, the velocity distribution profile becomes increasingly important and temporally disperses the MUAPs that constitute the M-Wave (c.f. Figure 7). This distance-
dependent temporal dispersion has been utilized by several investigators to calculate the velocity distributions of peripheral nerves (Barker et al., 1975; Cummins et al., 1979).

Our interest in these approaches lies in the possibility that by examining M-Waves that are elicited at various distances from the recording site, we can predict what the M-Wave would look like at any given distance. In particular, if each MU has a unique motor conduction velocity associated with it, there will be a distance at which a sufficient level of temporal dispersion has occurred such that each MUAP is separated in time from all other MUAPs. With this being the case, the actual number of MUs in the muscle could then be exactly calculated by simply counting each discrete response.

This minimum distance ($D_{\text{min}}$) at which each MUAP can be observed in such a manner that it is separated in time from all of the remaining MUAPs depends on three factors:

1) the maximum motor conduction velocity ($V_{\text{max}}$),

2) the minimum difference in conduction velocity values between any two MUs ($\Delta V_{\text{min}}$), and

3) the maximum MUAP duration ($\tau_{\text{max}}$).

Specifically, we have that\textsuperscript{10}

$$D_{\text{min}} \geq \frac{\tau_{\text{max}} V_{\text{max}}}{\Delta V_{\text{min}}} \left( V_{\text{max}} - \Delta V_{\text{min}} \right)$$

Assuming the maximum MUAP duration is 15 msec, we have calculated values of $D_{\text{min}}$ as a function of $V_{\text{max}}$ and $\Delta V_{\text{min}}$. These values are tabulated in Table 14.

\textsuperscript{10}The derivation of this relationship is given in Appendix 8.
Table 14: Minimum required stimulus site to recording site distance (in meters) in order to uniquely observe each MUAP.

<table>
<thead>
<tr>
<th>Maximum Velocity $V_{\text{max}}$</th>
<th>Minimum Required Distance at which $\Delta V_{\text{min}} = 1.00 \text{ m/s}$</th>
<th>$0.10 \text{ m/s}$</th>
<th>$0.01 \text{ m/s}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>60 m/s</td>
<td>53</td>
<td>539</td>
<td>5399</td>
</tr>
<tr>
<td>80 m/s</td>
<td>95</td>
<td>959</td>
<td>9599</td>
</tr>
<tr>
<td>100 m/s</td>
<td>149</td>
<td>1499</td>
<td>14999</td>
</tr>
</tbody>
</table>

As we can see, even for modest values of $V_{\text{max}}$ and $\Delta V_{\text{min}}$, large distances are required in order to achieve the desired signal separation. While such distances are anatomically impossible, we may be able to accomplish the equivalent through the use of system analysis techniques. In particular, the nerve/muscle system can be viewed as a number of parallel subsystems consisting of motor neurons and their constituent muscle fibers (Figure 29). Treating the neural components as pure time delays and assuming that the system is linear, traditional system analysis methods can be employed to retrieve the system response function of the nerve/muscle system. Given this function, it would then be a trivial matter of evaluating the response for any given distance. In addition, the system response function could be used to establish the conduction velocity distribution of the neural component of the system (Lee and Milsum, 1971; Williams, 1972).
Figure 29: A schematic depiction of the nerve/muscle system illustrating its parallel architecture.
7.1.2 Theoretical Development

Consider the simple delay system shown in Figure 30a. Assuming that \( x(t) \) is delayed by \( \tau \) seconds, we have that

\[
y(t) = x(t-\tau) \quad [25]
\]

Designating \( h(t) \) to be the system response function representing the delay, \( y(t) \) can also be expressed as follows

\[
y(t) = x(t) * h(t) \quad [26]
\]

where \( * \) denotes the convolution operation. Hence we have that

\[
h(t) = \delta(t-\tau) \quad [27]
\]

where \( \delta \) represents the Dirac delta function.

\[\begin{array}{cc}
\text{x(t)} & \rightarrow \text{Delay} \rightarrow \text{y(t)} \\
\end{array}\]

\[\begin{array}{cc}
h(t) & \rightarrow \text{time} \\
\end{array}\]

\begin{figure}[h]
\centering
\begin{subfigure}{0.4\textwidth}
\centering
\includegraphics[width=\textwidth]{delay_system}
\caption{a) A system consisting of a simple delay.}
\end{subfigure} \hspace{0.5cm}
\begin{subfigure}{0.4\textwidth}
\centering
\includegraphics[width=\textwidth]{delay_response}
\caption{b) the corresponding system response function.}
\end{subfigure}
\caption{Figure 30: a) A system consisting of a simple delay. b) the corresponding system response function.}
\end{figure}
Let us now extend this discussion to a situation in which we are dealing with a multiple-input/multiple-output system, as shown in Figure 31a. The expression for the $j$th output is given by the following:

$$y_j = x_j \cdot h_j = x_j \cdot \delta(t-\tau_j)$$ \[28\]

Such a system may be used to represent signal transmission in a nerve, which basically consists of a number of parallel delay pathways, with the delay for any given pathway being a function of the distance between the two recording sites and the velocity with which the signal is conducted.

Summing the individual response functions yields the following expression:

$$h_s = \sum_{j=1}^{N} h_j = \sum_{j=1}^{N} \delta(t-\tau_j)$$ \[29\]

Assuming that each system response function is unique (i.e. that each delta function is uniquely located in time), the summed system response function $h_s(t)$ would simply consist of $N$ delta functions located at the appropriate delays. This being the case, the number of MUs in the muscle could be determined by simply counting the number of delta functions comprising the summed system response function (that is, we would not even have to predict the response that would be observed at a distance at which each MUAP is separated in time from all other MUAPs).
Figure 31: a) A parallel system consisting of a number of delay functions. b) The corresponding system response functions for each delay channel. c) The summed system response function.
Unfortunately, the \( x_i(t) \) and \( y_j(t) \) signals (and hence the \( h_j(t) \) signals) are generally unknown to us. Hence, we can not determine \( h_j(t) \) in the manner outlined above. However, we may be able to approach this problem in a different manner. Specifically, we can readily stimulate the nerve supra-maximally at two different sites along its length (\( d_1 \) and \( d_2 \)), and record the associated maximal muscle potentials, or M-Waves (\( m_{w1}(t) \) and \( m_{w2}(t) \), respectively), as shown in Figure 32. We can then calculate the system response function relating these two M-Waves, i.e.

\[
m_{w2}(t) = m_{w1}(t) * h_j(t)
\]  

[30]

If we are dealing with a linear system, we have that

\[
h_j(t) = \sum_{j=1}^{N} h_j(t) = h_v(t)
\]  

[31]

Hence, assuming that the system is linear, we can use this approach to calculate the number of MUs in the muscle.

In order to be able to justify the linearity assumption required by this approach, we have to develop a model for the generation of the M-Wave responses. This model is shown in Figure 33a. Specifically, each MU is represented by three components: an impulse generator (which represents the cell body of the motor neuron), a delay function (which represents conduction along the nerve fiber), and a MUAP generator (which represents the summed response of the individual muscle fiber action potentials as observed at the recording site).

In the case of voluntary muscle activation, the input to each MU (\( u_j(t) \)) is generally modeled in terms of a white noise process. When the amplitude of this noise exceeds a certain threshold, the impulse generator is triggered. Since we are employing supra-maximal electrical stimulation to
activate the muscle, Figure 33a can be modified as shown in Figure 33b. This latter figure reflects the fact that for supra-maximal stimulation, the motor neurons are all activated concurrently.

Figure 32: A schematic depiction of the recording configuration.
Figure 33: a) Model for the generation of the electromyogram for the case of voluntary muscle activation. b) The associated model for the case in which supra-maximal external electrical stimulation is employed.
The model shown in Figure 33b can be used to derive the signals that would be observed in response to supra-maximal stimulation at any site along the nerve. Specifically, if the nerve is stimulated at distances $d_1$ and $d_2$, we have that

$$m_{w_1}(t) = \sum_{j=1}^{N} a_j(t - \frac{d_1}{v_j}) \quad [32]$$

and

$$m_{w_2}(t) = \sum_{j=1}^{N} a_j(t - \frac{d_2}{v_j}) \quad [33]$$

where $a_j(t)$ is the $j$th MUAP and $v_j$ is its associated conduction velocity. By evaluating equation [30] in the frequency domain, i.e.

$$MV_J(f) = MW_J(f) H_c(f) \quad [34]$$

we eliminate the convolution operation. Utilizing the transform pair

$$a(t-\Delta t) \leftrightarrow A(f) e^{-i2\pi f \Delta t} \quad [35]$$

equations [32] and [33] can be transformed into the frequency domain, yielding

$$H_c(f) = \left( \sum_{j=1}^{N} \frac{A_j(f) e^{-i2\pi f d_j/v_j}}{\sum_{j=1}^{N} A_j(f) e^{-i2\pi f d_j/v_j}} \right) \quad [36]$$
Hence

\[ h_s(t) = \mathcal{F}^{-1}(H_s(f)) = \mathcal{F}^{-1}\left( \frac{\sum_{j=1}^{N} A_j(f) e^{-i2\pi f d_{j}/v_j}}{\sum_{j=1}^{N} A_j(f) e^{-i2\pi f d_{j}/v_j}} \right) \]  \hspace{1cm} [37]

where \( \mathcal{F}^{-1}\) represents the inverse Fourier transform operation. As we can see, \( h_s(t) \) is a function of the individual MUAP waveforms. In order to eliminate this dependence, we introduce a simplifying assumption. Specifically, let us assume that the individual MUAPs are similar in shape to one another, and only differ by a scalar weighting factor \( w_j \). Hence, the expressions for \( mw_1(t) \) and \( mw_2(t) \) can be written as follows:

\[ mw_1(t) = \sum_{j=1}^{N} w_j a(t - \frac{d_j}{v_j}) \]  \hspace{1cm} [38]

\[ mw_2(t) = \sum_{j=1}^{N} w_j a(t - \frac{d_j}{v_j}) \]  \hspace{1cm} [39]

Hence, the expression for \( h_s(t) \) becomes

\[ h_s(t) = \mathcal{F}^{-1}\left( \frac{\sum_{j=1}^{N} w_j A_j(f) e^{-i2\pi f d_{j}/v_j}}{\sum_{j=1}^{N} w_j A_j(f) e^{-i2\pi f d_{j}/v_j}} \right) = \mathcal{F}^{-1}\left( \frac{\sum_{j=1}^{N} w_j e^{-i2\pi f d_{j}/v_j}}{\sum_{j=1}^{N} w_j e^{-i2\pi f d_{j}/v_j}} \right) \]  \hspace{1cm} [40]
While this eliminates the dependence of $h_c(t)$ on the shape of the individual MUAPs, it is still difficult to interpret equation [40]. Hence, we simplify matters further by assuming that $d_i$ equals 0. This reduces the above to

$$h_c(t) = e^{-j\pi \sum_{j=1}^{N} \omega_j / \nu_j}$$

Since the denominator in equation [41] is a constant, we can re-write the above as follows:

$$h_c(t) = \frac{1}{w_{sum}} \sum_{j=1}^{N} w_j \delta(t - \frac{d_j}{\nu_j})$$

Looking at equation [42], we see that the system response function consists of a number of delta functions located at the appropriate delays. Thus, assuming that all of the conduction velocities are different, the number of MUs in the muscle can readily be calculated by simply counting the number of delta functions present in this response function.
7.1.3 Practical Considerations

To determine the practicability of this approach, we now examine the consequences of the simplifying assumptions and limitations associated with measurement considerations.

**Sampling Frequency Considerations**

As we have stated throughout our discussion, it is assumed that the conduction velocity of each MU is different from that of the remaining MUs. This assumption is necessary in order to be able to detect the contribution of each MU. Given that this is the case, we can specify the minimum sampling frequency \( f_s \) required to be able to distinguish between delta functions associated with MUs with similar conduction velocities. Specifically, we have that\(^{11}\)

\[
f_s \geq \frac{2D \Delta V_{\min}}{(V-\Delta V_{\min})V}
\]

where \( V_{\text{max}} \) is the maximum motor conduction velocity, \( \Delta V_{\min} \) is the minimum difference in conduction velocity values between any two MUs, and \( D \) is the distance between the two stimulation sites. We have calculated the minimum required sampling frequency as a function of the first two parameters for the case in which the distance between the two stimulus sites is 40 cm. The results can be found in Table 15.

<table>
<thead>
<tr>
<th>Maximum Velocity ( V_{\text{max}} )</th>
<th>Minimum Sampling Frequency for ( \Delta V_{\min} = )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.00 m/s</td>
</tr>
<tr>
<td>60 m/s</td>
<td>17,700</td>
</tr>
<tr>
<td>80 m/s</td>
<td>31,600</td>
</tr>
<tr>
<td>100 m/s</td>
<td>49,500</td>
</tr>
</tbody>
</table>

\(^{11}\)The derivation of this relationship is given in Appendix 8.
Most commercially available A/D converters have maximum sampling frequencies on the order of 1 MHz, restricting us to analyzing systems in which $\Delta V_{mn}$ is on the order of 0.10 m/s. However, due to a lack of detailed information concerning the velocity distribution of peripheral nerves, it is difficult to say whether or not 1 MHz is sufficient to detect the contribution of each MU.

It should be noted that $h_c(t)$ as given equation 41 is not identical to the sum of the individual system response functions for each MU (i.e. $h_s(t)$). Specifically, each term in equation 41 is associated with a weighting term, whereas all of the delta functions comprising $h_s(t)$ have identical amplitudes of magnitude one. This has important ramifications concerning the sampling frequency that must be employed. Consider the simple case in which we have 3 MUs which are associated with motor neuron conduction velocities of 50, 60 and 80 m/s, and MUAP amplitudes of 20, 30 and 40 $\mu$V, respectively. Figure 34 shows the plots of $h_s(t)$ and $h_c(t)$ for the case in which $d_1 = 0$ cm and $d_2 = 40$ cm. As we can see, in both cases, the number of MUs can be simply obtained by counting the number of delta functions that are present. Now, let us assume that we have 3 MUs with motor neuron conduction velocities of 50, 50.1 and 80 m/s and the same MUAP amplitudes as above. The associated $h_s(t)$ and $h_c(t)$ functions are shown in Figure 35\textsuperscript{12}. In this case, the number of MUs can not be obtained by simply counting the number of constituent delta functions. However, there is a way of obtaining the number of MUs from $h_s(t)$. Specifically, since the contribution of each MU to $h_s(t)$ is a delta function of unity amplitude, we can simply sum the amplitudes of the observed delta functions. Unfortunately, this procedure can not be extended to the analysis of $h_c(t)$ since each term is weighted by the relative amplitude of the particular MUAP. Hence, we require that the sampling frequency be sufficiently high such that the contribution of each MU (namely an appropriately weighted delta function) can be detected. Interestingly, there is a positive aspect to this. Specifically, the amplitude distribution of the delta

\textsuperscript{12}In deriving these functions, the sampling frequency was specified such that we could not distinguish between two MUs with velocities of 50 and 50.1 m/s.
functions observed in $h_c(t)$ yields the MUAP amplitude distribution. Hence, this method not only provides the MU estimate, but also the amplitudes of these MUs!

**Figure 34:** A comparison of a) $h_c(t)$ and b) $h_c(t)$ for the case in which the conduction velocities of the three motor neurons are different.

**Figure 35:** A comparison of a) $h_c(t)$ and b) $h_c(t)$ for the case in which two of the MUs have conduction velocities which are nearly identical.
Examination of the Requirement that $d_i = 0$

In arriving at equation 41, we simplified matters by assuming that $d_i = 0$. This implies that the muscle must be directly stimulated. This may be difficult using surface stimulation where large stimulus intensities would have to be employed to ensure that the entire muscle is uniformly activated. In addition, it would be difficult to eliminate the stimulus artifact from the evoked muscle potential. The same difficulties would also be associated with an invasive micro-stimulation based approach.

We examined the influence of the value of $d_i$ on the system response function by simulating a simple two MU muscle system in which the MUAPs and their associated conduction velocities are known. For the case in which $d_i = 0$ (Figure 36), the system response function consisted of two appropriately delayed delta functions, as expected. When $d_i$ was increased to 10 mm, each delta function was replaced by a decaying sequence of delta functions\(^\text{13}\) (Figure 37). Hence, the system is only linear for cases in which $d_i = 0$.

\(^{13}\)The theoretical derivation for the system response function for cases in which $d_i \neq 0$, given in Appendix 9, predicts a sequence of delta functions.
Figure 36: The response function calculated for the case in which we have two MUs ($v_1=50$ m/s, $v_2=100$ m/s, $w_1=0.75$ and $w_2=1.0$) and the nerve is stimulated at distances of $d_1=0$ mm and $d_2=300$ mm. a) and b) show the constituent MUs. c) and d) show the M-Wave responses calculated at $d_1$ and $d_2$, respectively. e) shows the desired response function and f) shows the calculated response function.
Figure 37: The response function calculated for the case in which we have two MUs \( (v_1=50 \text{ m/s, } v_2=100 \text{ m/s, } w_1=0.75 \text{ and } w_2=1.0) \) and the nerve is stimulated at distances of \( d_1=10 \text{ mm and } d_2=310 \text{ mm.} \) a) and b) show the constituent MUs. c) and d) show the M-Wave responses calculated at \( d_1 \) and \( d_2 \), respectively. e) shows the desired response function and f) shows the calculated response function.
Examination of the Requirement that the MUAPs are Similarly Shaped

In arriving at equation 41, we also assumed that the MUAPs were similarly shaped and only differed in terms of a weighting factor. This assumption is generally not valid. MUAPs recorded from normal muscles generally exhibit some shape inhomogeneity, particularly if they are recorded with needle electrodes. While surface recorded MUAPs exhibit less variation, shape differences are nonetheless present. In neurogenic muscles, the shape inhomogeneity is even greater as the denervation/reinnervation process results in polyphasic potentials.

We evaluated the influence of shape inhomogeneity by again using our simple two MU muscle model. As we have already seen, with similarly shaped MUAPs, the system response function simply consists of two delta functions. When the shape of one of the MUAPs is altered, the system response function is much more complicated (Figure 38). Therefore, for all practical purposes, this method can not be used when muscle potentials are employed.
Figure 38: The response function calculated for the case in which we have two MUs which are not identically shaped ($v_1=50$ m/s, $v_2=100$ m/s) and the nerve is stimulated at distances of $d_1=0$ mm and $d_2=300$ mm. a) and b) show the constituent MUs. c) and d) show the M-Wave responses calculated at $d_1$ and $d_2$, respectively. e) shows the desired response function and f) shows the calculated response function.
7.1.4 Extending this Method to Incorporate NFAPs

As we have seen above, the system response method examined here can not be employed unless the constituent signals are identically shaped. While MUAPs do not fulfill this criteria, NFAPs do. Specifically, as we have stated earlier, it is generally agreed that NFAPs are similar in shape to one another and only differ by a velocity-dependent weighting factor. In addition, it may be possible to both stimulate and record at the same site (i.e. satisfying the $d_i=0$ requirement) by employing micro-stimulation techniques.

Unfortunately, since the nerve consists of literally thousands of nerve fibers, extremely high sampling frequencies would have to be employed in order to yield a response function in which the contribution of each fiber (namely a spike located at the appropriate delay) could be readily resolved. Even if this could be achieved, we would again be left with the problem of distinguishing the alpha motor neurons from the remaining fibers.
7.2 A Multiple Stimulation Site Based Approach

7.2.1 Theoretical Development

Let us now re-examine the approach we developed in the previous section. Specifically, let us look at the case in which the muscle consists of two MUs. The signals that would be observed in response to stimulation at distances \( d_1 \) and \( d_2 \) are

\[
mw_1(t) = a_1(t) \cdot \delta(t - \frac{d_1}{v_1}) + a_2(t) \cdot \delta(t - \frac{d_2}{v_2})
\]

[45]

and

\[
mw_2(t) = a_1(t) \cdot \delta(t - \frac{d_1}{v_1}) + a_2(t) \cdot \delta(t - \frac{d_2}{v_2})
\]

[46]

respectively. If we knew the values of \( v_1 \) and \( v_2 \), the above system could be readily solved in a least squares sense to yield \( a_1(t) \) and \( a_2(t) \). While in practice we do not explicitly know these velocity values, we do know the range in which they fall. We can therefore partition this range into a number of discrete values and evaluate the system of equations at these values. The solutions can be tested by predicting the M-Wave response that would be observed at a third distance \( d_3 \). The sum-of-squares error between the predicted signal and that actually observed can be used to determine the correct solution. In the ideal case in which no noise is present, the correct solution will be associated with a sum-of-squares error of zero. When noise is present, the correct solution will be associated with a sum-of-squares error that is smaller than that produced by other solutions.

Theoretically, this approach can be readily extended to count the number of MUs in a muscle. Specifically, let us assume that the nerve can be supra-maximally stimulated at \( M \) sites along its length, where \( M \) is greater than the number of MUs in the muscle. Let us further assume that MUs with identical conduction velocities are associated with MUAPs that are linearly independent from one another. The latter is an essential requirement to insure the uniqueness of the solution.
We then start off by assuming that the muscle consists of two MUs, and obtain these MUs using the method discussed above. The retrieved MUAPs are used to predict the M-Wave response observed at some reference position, and the sum-of-squares error between the predicted and observed signals is calculated. The assumed number of MUs is continually increased until there is no significant decrease in the sum-of-squares error. Specifically, the error will continue to decrease until the correct number of MUs has been specified. Increasing the assumed number of MUs beyond the actual number will yield MUAP sub-units whose linear combination yields the original MUAP, and hence will not decrease the sum-of-squares error.

7.2.2 Practical Considerations
A simulated example of this approach for the case of two MUs is illustrated in Figure 39. Unfortunately, the requirement that the number of stimulation sites be larger than the number of MUs in the muscle makes this approach impractical. Specifically, the nerve may have to be stimulated at possibly hundreds of different sites. Not only would this be time consuming, but hundreds of repeated maximal stimulations would not be acceptable to patients. In addition, the method is computationally intensive, as large systems of equations must be solved.
Figure 39: Illustration of the decomposition procedure used to retrieve the constituent MUAPs from the observed M-Wave response. In this case, the muscle consists of two MUs, whose associated MUAPs are shown in a). The M-Waves observed in response to stimulation at various sites along the nerve are given in b). Gaussian noise has been added to these M-Waves to examine the robustness of this method. The estimated MUAPs are shown in c), with the re-constructed M-Waves shown in d).
7.3 A Modified Tauberian Approximation Method
7.3.1 Theoretical Development

In Tauberian approximation (Weiner, 1933), a signal is represented as a linear combination of shifted versions of a single (known) basis signal, i.e.

\[ y(t) = \sum_{i=1}^{N} n_i x(t-\tau_i) \]  \[47\]

where \( x(t) \) is the basis signal, \( y(t) \) is the signal being represented in terms of the shifted basis signals, \( N \) is the number of shifted signals required to represent \( y(t) \), and \( n_i \) and \( \tau_i \) are the unknown weighting and delay parameters, respectively. This approach can be modified to approximate NFAPs. Specifically, the expression for the maximal observable nerve potential is given by the following

\[ f(t) = \sum_{i=1}^{N} a_i(t - \tau_i) = \sum_{i=1}^{N} a_i(t - d/v_i) \]  \[48\]

where \( f(t) \) is the maximal nerve potential, \( N \) is the number of constituent nerve fibers, \( a_i(t) \) is the \( i \)th NFAP, \( v_i \) is its associated conduction velocity, and \( d \) is the distance between the stimulating and recording sites. Since the individual NFAPs are generally similar in shape and only differ in terms of a velocity-dependent weighting factor, equation \[48\] can be re-written as follows:

\[ f(t) = \sum_{i=1}^{N} w(v_i) a(t - d/v_i) \]  \[49\]

where \( w(v) \) is the weighting factor and \( a(t) \) is the representative NFAP. As stated in Chapter 2, current estimates for the weighting factor range from a linear dependence to one in which its amplitude varies as the square of the velocity (Barker et al., 1975; Cummins et al., 1979).
As it stands, we cannot solve equation [49] since we do not \textit{a priori} know the value of \( N \). Fortunately, equation [49] can be approximated by dividing the conduction velocity range into a finite number of velocity bins, as follows:

\[
  f(t) = \sum_{j=1}^{M} n_j \ w(v_j) \ a(t - d/v_j) \quad [50]
\]

where \( M \) is the number of velocity bins, \( v_j \) is the mean velocity of the \( j \)th velocity bin and \( n_j \) is the number of nerve fibers with conduction velocities falling within the range of this bin\(^{14}\). Since the \( v_j \) values are now known, the only unknown parameters are the \( n_j \) values and the \( a(t) \) waveform. Strictly speaking, equation [50] does not represent a Tauberian approximation. For this reason, we call the expression given by equation [50] a modified Tauberian approximation.

Given \( f(t) \), \( a(t) \), \( M \), \( d \), and the \( v_j \) and \( w(v_j) \) values, least-squares methods can be used to solve equation [50] to yield the \( n_j \) values. The number of constituent nerve fibers is then simply given by

\[
  N = \sum_{j=1}^{M} n_j \quad [51]
\]

\(^{14}\)As the velocity bin width approaches 0 in the limit, equation 50 reduces to equation 49.
7.3.2 Practical Considerations

The main problem with this approach is that $a(t)$ can not be readily calculated. Specifically, the NFAP associated with the activation of a single nerve fiber is extremely small and is generally below the noise levels of both the recording system and the environment. Therefore we have to estimate the signal using a modeling approach (Barker et al., 1979; Xiao et al., 1988). Unfortunately, in following this approach, it is necessary to know the distance between the nerve and the recording electrode since it plays a major role in determining the amplitude of the signal. It is difficult to obtain this information in a non-invasive manner.

There are also other difficulties associated with this seemingly simple approach. Firstly, we do not know the exact nature of the velocity dependent weighting factor. Secondly, as with other NFAP-based methods, we must face the difficult problem of having to distinguish the alpha motor fibers from the remaining fibers.

7.4 Discussion

Of the three methods presented in this chapter, the multiple stimulation site method is the least practical due to its requirement of a large number of stimulation sites. However, it may be worthwhile to examine the "basis" signals obtained when only a limited number of M-Waves are employed. Specifically, the latency and number of phases of the MUAPs are useful in detecting neurogenic disease processes.

The method based on evaluating the system response function is appealing in that only two maximal responses elicited at different distances along the nerve are required to generate an estimate. While the linearity requirements are generally not met using MUAPs, they can be satisfied when NFAPs are recorded. While extremely high sampling frequencies would have to be employed in order to resolve the delta functions associated with the propagation delay of each
nerve fiber, these frequencies could be achieved using currently available A/D boards. We would still be left with the problem of distinguishing the contribution of the alpha motor fibers from that of the other fibers. One way of approaching this problem would be to establish confidence intervals for the relative fraction of alpha motor fibers based on current anatomical data. In this way, an estimate range could be obtained. Advances in our understanding of the composition of peripheral nerves in terms of their constituent fiber groups would further increase the attractiveness of such an approach.

The modified Tauberian approximation approach also has much promise. Specifically, given the maximal NFAP, the representative NFAP \( a(t) \) and the velocity-dependent weighting function, the number of nerve fibers can be readily calculated. The major problem associated with this method is that of obtaining the representative NFAP. While the solution of this problem is outside the scope of our work here, it poses a fascinating challenge for other investigators in this field.
8. DISCUSSION

In this thesis, we have presented a new method for estimating the number of MUs in a muscle. This method, termed MUESA, represents a significant departure from current estimation methods. Specifically, while it is now well recognized that MUs are activated in a probabilistic manner in response to external electrical stimulation, there is no general consensus in terms of how to deal with the problems associated with such activation. In particular, in cases in which multiple MUs are undergoing probabilistic activation, successive increments in the muscle potential can not be simply interpreted in the context of the successive activation of single MUs. The majority of the researchers in this field have dealt with this issue in one of two different ways:

1) By assuming that the incremental increases do represent the activation of single MUs and then going back and testing for the probabilistic activation of multiple MUs using special "alternation" detection algorithms. These investigators view probabilistic activation in terms of a secondary phenomenon.

2) By stimulating the nerve at a number of different sites and only acquiring the first few MUs that are activated at each site. Since such MUs are generally activated in a sequential manner, these investigators circumvent the alternation phenomenon and only face the trivial problem of properly extracting the MUAPs from the first few muscle responses.

In contrast to these approaches, we started our research with the belief that there was information in the responses associated with alternating MUs. As a result, we developed a fundamentally new method termed MUESA in which we capitalize on probabilistic MU activation to obtain our representative MU sample. In this method, the nerve is subjected to a number of constant-intensity stimulus trains, and the resultant response sequences are then analyzed to yield the constituent MUAP(s). In general, if a stimulus train results in the probabilistic activation of n motor units, we can expect to see up to $2^n$ different responses, with each response representing a unique combination of active and/or inactive motor units. If all $2^n$ responses are
indeed observed, the decomposition of the observed response sequence into its constituent motor unit action potentials is trivial. For the majority of the cases in which the number of observed responses is not an integer power of 2, a novel decomposition method is employed to extract the constituent MUAP(s) from these incomplete sequences. The development of this decomposition method was based on a theoretical analysis of the probabilistic activation framework which showed that the relative activation rates of the motor units could be used to obtain the constituent MUAP(s).

The advantages of our method are as follows:

1) We eliminate the uncertainty associated with the interpretation of the observed muscle potentials. Specifically, by analyzing the muscle responses in the context of a probabilistic activation framework, we do not have to make the assumption that each successive muscle response represents the activation of a new MU. This is significant, given that many researchers recognize probabilistic MU activation (also termed alternation) to be one of the most serious problems associated with the original increment-counting technique and its derivatives.

2) By using constant intensity stimulus trains to activate the MUs, a certain level of control is exercised over the nerve/muscle system in that each stimulus train generally results in the probabilistic activation of a limited number of MUs. Constant intensity stimulus trains also allow us to examine the stationarity of the nerve/muscle system. Specifically, an underlying assumption of all of the estimation methods is that potentials generated by each MU do not vary during the course of the examination. However, in certain disease states, this assumption is not valid. For example, in ALS, the amplitude of the compound muscle potential (and hence of its constituent MUAPs) decreases in response to repetitive stimulation. Since MU estimation studies would not be amenable to the study of such muscles, it is important to be able to detect these non-stationarities. For the purposes of this investigation, we restricted our analysis to sequences which contained
eight or fewer unique responses, which effectively assured that the system remain
stationary during the course of the stimulation.

3) Since each response sequence is analyzed as a whole using a clustering procedure (as
opposed to a sequential analysis procedure such as the template matching technique),
we obtain classifications that are invariant to the input order of the responses.

To test this new approach we examined the EDB and thenar muscles in two different subject
groups:

1) a control group, which consisted of subjects with no reported neuromuscular deficits, and
2) a neurogenic group, which consisted of poliomyelitis subjects.

Our estimates indicate that the thenar muscles contain more MUs than the EDB, which is
consistent with the findings of other investigators. In terms of absolute numbers, the control
estimates we obtained are generally lower than those obtained by investigators employing the
increment counting technique and its derivatives, but are similar to those obtained by
investigators employing methods based on the spike triggered averaging based technique. It is
important to stress that in diagnostic terms, absolute estimates are not as important as the
relative difference between estimates obtained from normal and diseased muscles.\textsuperscript{15} In this
context, we found that the MU estimates obtained from the neurogenic group were significantly
lower than those obtained from the control group.

In addition to developing a new estimation method that addresses many of the problems
associated with other methods, we investigated several alternative estimation strategies that
move away from the sampling procedure inherent in all of the methods published to date. To our
knowledge, we are the first investigators to examine any such alternative strategies. Our

\textsuperscript{15}It should be noted that the number of MUs in a muscle can not be unequivocally confirmed
using existing histological methods.
investigation resulted in the development of the theoretical basis for three entirely new approaches:

1) a method based on analyzing the system response function obtained by comparing two maximal evoked muscle potentials,

2) a method based on the analysis of multiple maximal evoked muscle potentials elicited in response to supramaximal stimulation at multiple nerve sites, and

3) a method based on modeling the maximal evoked nerve potential in terms of a modified Tauberian approximation.

All of these methods capitalize on the parallel architecture of the nerve muscle system in which the motor neurons and muscle fibers can be viewed as pure delays and signal generators, respectively. While the implementation of these methods was beyond the scope of this thesis, they illustrate the alternative research avenues that are available and should be investigated in greater detail.
9. REFERENCES


APPENDIX 1. MODIFICATIONS TO THE ORIGINAL INCREMENT-COUNTING TECHNIQUE

Since the development of the increment-counting technique in 1971 by McComas and colleagues, a number of investigators have introduced various modifications. These modifications are discussed here.

In an effort to minimize the possibility of missing small amplitude MUAPs, Panayiotopoulos et al. (1974) used a technique in which they superimposed photographs of MUAPs. However, McComas (1977a) disputes the estimates that they obtained on the basis that the noise level in their measurements is greater than the increments between successive compound muscle potentials. Ballantyne and Hansen (1974, 1974a) tackled the same problem by employing a template-matching method in which potentials classified as being the same were averaged to form a representative MUAP template. This method marked the first time a computer was used to both acquire the data and assist in the analysis. Using this method, Ballantyne and Hansen (1974, 1974a, 1974b) were able to obtain normal MUEs for subjects with myasthenia gravis and Duchenne, limb girdle, facioscapulohumeral, and myotonic muscular dystrophies, whereas McComas and colleagues (1974) found reduced estimates.

Along the same lines as Ballantyne and Hansen, Jasechko and DeBruin (1987) introduced a new automatic implementation of the increment-counting technique. However, as with the method developed by Ballantyne and Hansen, this new implementation does not really address the limitations of the increment-counting technique.

Milner-Brown and Brown (1976) introduced two methods particularly aimed at reducing the alternation problem and obtaining a representative MU sample. Both of these techniques involve the delivery of a series of constant-intensity pulse trains to the nerve.
Method 1
A pulse train is delivered at an amplitude just sufficient to activate the first MU. The minimum, maximum, and predominant amplitudes of the recorded potentials are then calculated. These are referred to as $A_{\text{min}}(1)$, $A_{\text{max}}(1)$, and $A_{\text{pd}}(1)$, respectively. (The predominant amplitude is defined as the amplitude that is associated with the most number of occurrences). The stimulus level is then increased until a potential greater than $A_{\text{max}}(1)$ is obtained. This then serves as the new level at which the pulse train is delivered. Again, the minimum, maximum, and predominant potentials are calculated. This sequence is repeated a number of times. The differences between successive potentials are then taken to represent the additional recruitment of individual MUs if two criteria are satisfied. Namely, $A_{\text{pd}}(n+1) > A_{\text{pd}}(n)$ and $A_{\text{max}}(n+1) > A_{\text{max}}(n)$. The mean MUAP is then calculated by dividing the number of identified MUs into the amplitude of the compound muscle potential.

Method 2
This method utilizes the fact that the maximum number of possible amplitude combinations (observed as a series of unique responses) that $n$ MUs with overlapping thresholds can exhibit is $2^n$. Therefore, assuming that all of these possible combinations have been generated, the number of MUs is simply given by

$$n = \log_2(\text{number of responses})$$  \[1\]

Neither method 1 nor method 2 are commonly employed. Part of the reason for this may be the added complexity associated with these methods. Brown and Milner-Brown (1976) have suggested a simpler approach which is based on the observation that some MUs can be recruited before the alternation phenomenon comes into play. Therefore, by stimulating the nerve at multiple sites and recruiting only a few MUs at each site, a large representative MU sample
can be acquired. However, it is still unresolved as to whether or not the first few MUs are representative of the entire MU population.

Daube (1988) also developed an approach using constant intensity stimulus trains, but analyzes the resulting potentials in a completely different fashion. Specifically, he employs a statistical approach originally used to analyze the quantal release of acetylcholine (ACh) at the neuromuscular junction. In particular, assuming that each evoked is made up of MU responses which fire at random intervals according to a Poisson process, the mean MU amplitude can be calculated as follows (Katz and Melidi, 1972):

\[
\text{mean MUAP amplitude} = \frac{\text{variance of the response amplitudes}}{\text{mean response amplitude} - \text{minimum response amplitude}}
\]  

However, this approach makes the unfounded assumption that all of the MUs that are undergoing probabilistic recruitment have identical shapes and amplitudes. Daube has obtained estimates which are smaller than those generally obtained using the original increment-counting technique.

Stein and Yang (1990) introduced several modifications. Firstly, they replaced the surface stimulation protocol with an invasive, micro-stimulation protocol. This yielded estimates that were smaller than those they obtained using surface stimulation. The investigators speculated that these reduced estimates may have been caused by the preferential excitation of larger diameter axons (and hence larger MUs). That is, since the distance from the stimulating electrode to the nerve fiber is greatly reduced, fiber diameter plays an increased role in nerve fiber activation. They also evaluated the utility of using twitch tension as the measure of muscle output. This measure has a number of favorable qualities. Firstly, unlike the EMG measure which can be corrupted by the activity of distant muscles due to volume conduction, the tension should not be affected by the activity of these muscles. Secondly, it provides information about the contractile
properties of the MU. The major disadvantage with this approach is that an averaging procedure must be used to extract the responses. 500 sweeps were typically required for the extraction of each twitch. Interestingly, Stein and Yang found that the tension-based estimates were very similar to the EMG based measurements.
APPENDIX 2. ESTIMATES OBTAINED BY PUBLISHED METHODS

1. Nerve and Muscle Diseases

The motor unit essentially consists of four functional components: the cell body of the motor neuron, the axon of the motor neuron, the neuromuscular junctions, and the muscle fibers innervated by the motor neuron. Each one of these components is susceptible to disease. For example, Myasthenia Gravis is an autoimmune syndrome that affects ACh receptors and is limited to the neuromuscular junction. While, the majority of nerve and/or muscle unit diseases cause weakness and wasting of skeletal muscle, they may differ in terms of which component is primarily affected. It was in the nineteenth century that two different types of diseases were initially distinguished from one another through postmortem examination. Neurogenic diseases are characterized by pronounced changes in the nerve cell bodies (motor neuron diseases) or axons (peripheral neuropathies) with only minor changes in the muscle fibers; since motor and sensory axons run in the same nerves, peripheral nerve disorders usually elicit both sensory and motor deficits. Myopathic diseases, on the other hand, are characterized by the advanced degeneration of muscles, with little change in motor neurons or axons. However, this distinction between neurogenic and myopathic classifications is currently under much controversy in that some investigators believe that they might not be mutually exclusive disorders. In particular, Kugelberg and Welander (1956) found that patients with spinal muscular atrophy exhibited symptoms closely resembling muscular dystrophy. These "secondary myopathic changes", as they have been termed, have also been found in other chronic denervating disorders (Tyrer and Sutherland, 1961; Drachman, Murphy, Nigam and Hills, 1967). One of the more intriguing findings comes from Dubowitz (1969). Based on evidence from reinnervation studies, Dubowitz suggested that dystrophic muscle changes may be due to neural influences. McComas and colleagues (1971) seemed to verify this by their findings that Duchenne muscular dystrophy was associated with a loss of MUs. However, this is at odds with both the findings of other investigators who have found normal MU estimates in these muscles and also autopsy studies
showing normal complements of anterior horn cells. McComas and colleagues (1971) answered this criticism by stating histochemically normal neurons may function abnormally. This led to the development of a theory proposed by these investigators termed the *sick motoneurone hypothesis* (c.f. McComas, 1988). This theory suggests that a number of clinical disorders that had previously been regarded primarily as disorders of the muscle fiber are instead expressions of dysfunctional motor neurons. In particular, this theory states that there are three stages of motor neuron function: healthy, sick and dead. A motor neuron is regarded as healthy if it satisfies the following criteria:

1) it conducts impulses at normal rates along the axon,
2) it effectively transmits the excitation across the neuromuscular junction, and
3) it maintains all of the muscle fibers of the motor unit in a healthy condition.

A sick motor neuron, on the other hand, has difficulty maintaining satisfactory synaptic connection with its muscle fibers. Thus, axonal conduction velocity could be normal, but the ability to acquire previously denervated muscle fibers is impaired. Finally, a dead motor neuron is one in which no influence is exerted on the muscle fibers.

Several features can be used to distinguish between MUAPs recorded from normal, neurogenic and myopathic muscles. The three most characteristic features are the amplitude, duration and number of phases. In general, MUAPs recorded from neurogenic muscles are characterized by larger amplitudes, longer durations and a greater number of phases than their normal counterparts. These changes are a consequence of the reinnervation process that usually accompanies the neurogenic disease process. That is, muscle fibers originally innervated by motor neurons which subsequently die are reinnervated by the remaining healthy motor neurons. Since the number of muscle fibers which make up these MUs increases, their associated MUAP amplitudes increase in amplitude. The increased duration and greater number of phases are explained on the basis that the reinnervation process results in an increased degree of temporal dispersion of the individual muscle fiber action potentials. Figure A1 compares the typical MUAPs
recorded from normal and neurogenic muscles. MUAPs recorded from myopathic muscles on the other hand, are generally characterized by smaller amplitudes and shorter durations than normal MUAPs, reflecting the decrease in the number of constituent muscle fibers for these MUs.

It is important to note that there is significant variation in these MUAP features even in normal subjects. Additionally, these features depend on a number of factors including:

1) the shape, recording characteristics and positioning of the recording electrode,

2) the distances between the recording electrode and the constituent muscle fibers of the motor unit, and

3) the conductivity of the intermediate tissue.

**Figure A1:** A comparison between the MUAPs recorded from normal and neurogenic muscles.  
a) Normal MUAP  b) Neurogenic MUAP
2. Estimates Obtained from Normal Muscles

Table A1 lists the estimates obtained from normal muscles by the various approaches. The increment-counting technique and its variations have been used mainly to study the small muscles of the hand (i.e. thenar and hypothenar muscle groups), and the EDB. Of these muscles, the latter has been studied most extensively (Ballantyne and Hansen, 1974, 1974a; Bolton et al., 1979; McComas et al., 1971a; Panayiotopoulos and Scarpalezos, 1974; Panayiotopoulos and Scarpalezos, 1975). The results obtained by Panayiotopoulos and colleagues and also Brandstater et al. notwithstanding, there seems to be a good agreement between the results obtained by different investigators employing the ICT. The higher estimates obtained by Panayiotopoulos and colleagues have been questioned by other investigators. Specifically, Panayiotopoulos et al. used photographic techniques to discriminate between increments smaller than the background noise level. However, signal variations caused by changes in the stimulus artifact and/or instrumentation noise may have been incorrectly categorized as incremental responses (Milner-Brown and Brown, 1976). This would lead to falsely low mean MU potential amplitudes, and hence high MU estimates. It should be pointed out that there is some histological (Jennekens et al., 1976) and electrophysiological (Roselle and Stevens, 1973) evidence that challenges the use of the EDB as a representative muscle. In particular, the evidence tends to suggest that denervation and reinnervation normally occur in this muscle.

The thenar muscle group has also been extensively studied (Brown, 1972; Campbell et al., 1973; deWeerd, 1984; Jasechko and DeBruin, 1987; Milner-Brown and Brown, 1976; Sica et al., 1974; Stein and Yang, 1990). In this case, the estimates are distributed over a larger range. The results for the hypothenar muscle groups correspond well (Carleton and Brown, 1978; Milner-Brown and Brown, 1976; Sica et al., 1974).
Other muscles such as the Soleus (McComas et al., 1974), Abductor Pollicus Longus (APL) (Defaria and Toyonaga, 1978), and the facial muscles (Debeke, 1982) have been investigated. However, it is difficult to assess these estimates since the investigations for each muscle have mostly been carried out by a single research group. For example, McComas and colleagues (1974, 1977, 1978) have conducted multiple evaluations of the soleus muscle, obtaining good correspondence between the results. However, other investigators have yet to evaluate this muscle.

The spike-triggered averaging based technique was originally employed to examine the biceps-brachialis (Brown et al., 1988; Strong et al., 1987). Being a larger muscle than those typically investigated with the increment-counting technique and its analogs, it is not surprising that larger MU estimates have been obtained.

The spike-triggered averaging based technique has recently been employed to evaluate the EDB (Barkhaus et al., 1990) and thenar muscles (Stein and Yang, 1990). These investigators found significantly lower estimates than those typically obtained for these muscles.

The use of the Macro-EMG based technique has been limited, with only the tibialis anterior being examined to date (deKoning et al., 1988).
<table>
<thead>
<tr>
<th>Investigators</th>
<th>Technique</th>
<th>Muscle</th>
<th>Estimates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ballantyne and Hansen (1974)</td>
<td>Modified Increment Counting Technique</td>
<td>EDB</td>
<td>197±49 (39)</td>
</tr>
<tr>
<td>Panayiotopoulos et al. (1975)</td>
<td>Modified Increment Counting Technique</td>
<td>EDB</td>
<td>411±125 (9)</td>
</tr>
<tr>
<td>McComas et al. (1978)</td>
<td>Increment Counting Technique</td>
<td>EDB</td>
<td>212±67 (162)</td>
</tr>
<tr>
<td>Brandstater et al. (1989)</td>
<td>Increment Counting Technique</td>
<td>EDB</td>
<td>135±76 (20)</td>
</tr>
<tr>
<td>DeBruin et al. (1989)</td>
<td>Increment Counting Technique</td>
<td>EDB</td>
<td>248±75 (23)</td>
</tr>
<tr>
<td>Barkhaus et al. (1990)</td>
<td>Spike-Triggered Averaging Based Approach</td>
<td>EDB</td>
<td>48±10</td>
</tr>
<tr>
<td>Lee et al. (1975)</td>
<td>Spike-Triggered Averaging Based Approach</td>
<td>thenar</td>
<td>167±14 (5)</td>
</tr>
<tr>
<td>Milner-Brown and Brown (1976)</td>
<td>Constant-Intensity Stimulus Train Based Approach</td>
<td>thenar</td>
<td>261±116 (6)</td>
</tr>
<tr>
<td>McComas et al. (1978)</td>
<td>Increment Counting Technique</td>
<td>thenar</td>
<td>343±97 (115)</td>
</tr>
<tr>
<td>Daube (1988)</td>
<td>&quot;Quantal&quot; Approach</td>
<td>thenar</td>
<td>185±32</td>
</tr>
<tr>
<td>Brandstater et al. (1989)</td>
<td>Increment Counting Technique</td>
<td>thenar</td>
<td>267±121 (20)</td>
</tr>
<tr>
<td>DeBruin et al. (1989)</td>
<td>Increment Counting Technique</td>
<td>thenar</td>
<td>453±257 (22)</td>
</tr>
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<td>Stein and Yang (1990)</td>
<td>Increment Counting Technique</td>
<td>thenar</td>
<td>170±62 (10)</td>
</tr>
<tr>
<td>Stein and Yang (1990)</td>
<td>Increment Counting Technique with</td>
<td>thenar</td>
<td>135±27 (10)</td>
</tr>
<tr>
<td></td>
<td>Micro-Simulation (EMG based)</td>
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<td></td>
</tr>
</tbody>
</table>

16Numbers in parentheses refer to the number of subjects examined.
Table A1 continued: Control estimates for various muscles.

<table>
<thead>
<tr>
<th>Investigators</th>
<th>Technique</th>
<th>Muscle</th>
<th>Estimates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stein and Yang</td>
<td>Increment Counting Technique with Micro-Simulation (tension based)</td>
<td>thenar</td>
<td>130±39</td>
</tr>
<tr>
<td>(1990)</td>
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<td></td>
<td>(10)</td>
</tr>
<tr>
<td>Stein and Yang</td>
<td>Spike-Triggered Averaging Based Approach (EMG based)</td>
<td>thenar</td>
<td>122±38</td>
</tr>
<tr>
<td>(1990)</td>
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<td></td>
<td>(10)</td>
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<tr>
<td>Stein and Yang</td>
<td>Spike-Triggered Averaging Based Approach (tension based)</td>
<td>thenar</td>
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<td>(1990)</td>
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<td>(10)</td>
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<tr>
<td>Brown et al.</td>
<td>Spike-Triggered Averaging Based Approach</td>
<td>biceps-brachialis</td>
<td>911±254</td>
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<td>(1988)</td>
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<td></td>
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</tr>
<tr>
<td>DeBruin et al.</td>
<td>Increment Counting Technique</td>
<td>biceps-brachialis</td>
<td>264±109</td>
</tr>
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<td>(1989)</td>
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<td>(21)</td>
</tr>
</tbody>
</table>

3. Estimates in Dystrophic Muscles

To date, all of the estimates obtained in dystrophic patients have been acquired using the increment-counting technique and its variations.

Duchenne Dystrophy

There has been some controversy concerning the number of MUs associated with Duchenne Dystrophy. In a study conducted on the EDB, McComas and Colleagues (1971d) found a decreased number of MUs. Further studies conducted on the thenar, hypothenar, and soleus muscles showed similar results (McComas et al., 1974), however the hypothenar estimates were not as reduced as those in the other muscles. Ballantyne and Hansen (1974) and Panayiotopoulos et al. (1974), on the other hand, have found normal MU numbers in the EDB. As pointed out above, the results of the latter investigators may be somewhat questionable. Additionally, neither of the above studies were as extensive as those carried out by McComas and colleagues. Finally, subsequent studies carried out by McComas and colleagues again found reduced MU numbers (1977).
Myotonic Dystrophy

Unlike the findings in Duchenne muscular dystrophy, there is no controversy concerning the estimates found in myotonic dystrophy. In particular, losses have been consistently found in the EDB (Ballantyne and Hansen, 1974b; McComas et al., 1971b; Polgar et al., 1972), the thenar (McComas et al., 1974; 1978) and the soleus (McComas et al., 1974; 1978) muscles. However, as in Duchenne muscular dystrophy, MU losses were less prominent for the hypothenar muscle group. It is interesting to note that the "curious sparing" of the hypothenar muscle groups is also a feature of peripheral axonal neuropathies (McComas, 1988).

Limbgirdle and Facioscapulohumeral Dystrophies

As with the findings in Duchenne muscular dystrophy, controversy exists over estimates found in these disorders. Sica et al. (1971) found reduced estimates in the EDB, while Ballantyne and Hansen (1974) and Panayiotopoulos and Scarpalezos (1975) found normal values. A subsequent study carried out by McComas and colleagues (1974) found reduced values in the thenar, hypothenar, soleus and EDB muscles. Again, it should be noted that the studies by Sica, McComas and colleagues encompassed a larger number of patients, and are therefore potentially more reliable.

Miscellaneous Dystrophies and Myopathies

Panayiotopoulos et al (1974) found normal estimates in the EDB muscles of two patients with Becker type muscular dystrophy. McComas (1988) also found normal estimates in the EDB of three patients with Becker dystrophy, however the thenar estimates were reduced in two of the subjects.

Elder et al (1987) found reduced EDB and thenar estimates in an infant with centronuclear myopathy. Upton et al. (1973) also found reduced EDB and thenar estimates in a patient with McArdles syndrome, with the hypothenar estimate being only slightly reduced.
<table>
<thead>
<tr>
<th>Investigator</th>
<th>Muscle</th>
<th>Control</th>
<th>Duchenne</th>
<th>Myotonic</th>
<th>Limb-Girdle</th>
</tr>
</thead>
<tbody>
<tr>
<td>McComas et al. (1971a,b)</td>
<td>EDB</td>
<td>199±60</td>
<td>Decreased</td>
<td>(41)</td>
<td>(12)</td>
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<td></td>
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<td>(17)</td>
<td></td>
<td></td>
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<tr>
<td>McComas et al. (1971d)</td>
<td>EDB</td>
<td>195±59</td>
<td>53±38</td>
<td>(47)</td>
<td>(19)</td>
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<tr>
<td>McComas et al. (1971e,f)</td>
<td>EDB</td>
<td>199±60</td>
<td>53±38</td>
<td>75±47</td>
<td>65±41</td>
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<td>(19)</td>
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<td>Polgar et al. (1972)</td>
<td>EDB</td>
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<td>Decreased</td>
<td>(17)</td>
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<tr>
<td>McComas et al. (1974)</td>
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<td>208±63</td>
<td>79±42</td>
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<td>(10)</td>
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<td>Ballantyne and Hansen (1974)</td>
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<td>197±49</td>
<td>191±62</td>
<td>80±46</td>
<td>228±56</td>
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<td>(12)</td>
<td>(12)</td>
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<tr>
<td>Panayiotopoulos et al. (1974)</td>
<td>EDB</td>
<td>370±107</td>
<td>295±131</td>
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<td>Panayiotopoulos et al. (1975)</td>
<td>EDB</td>
<td>411±125</td>
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<td>380±74</td>
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<tr>
<td>McComas et al. (1977)</td>
<td>EDB</td>
<td>206±65</td>
<td>68±46</td>
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<td>(50)</td>
<td>(50)</td>
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<tr>
<td>McComas et al. (1978)</td>
<td>EDB</td>
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<td>(162)</td>
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<td>(106)</td>
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<td>McComas et al. (1974)</td>
<td>thenar</td>
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<td>190±109</td>
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<td>(18)</td>
<td>(9)</td>
<td>(7)</td>
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<td>McComas et al. (1977)</td>
<td>thenar</td>
<td>401±111</td>
<td>137±54</td>
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<td>(23)</td>
<td>(22)</td>
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<tr>
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<td>thenar</td>
<td>343±97</td>
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<td>(115)</td>
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<td>(41)</td>
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<td>McComas et al. (1974)</td>
<td>hypothenar</td>
<td>380±79</td>
<td>244±80</td>
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<td>(10)</td>
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<td></td>
<td>(15)</td>
<td>(21)</td>
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</tbody>
</table>

17 Numbers in parentheses refer to the number of subjects examined.
4. Estimates in Neurogenic Disorders

Amyotrophic Lateral Sclerosis (ALS)

Studies have consistently shown reduced numbers of MUs in patients with ALS. In particular, using an analog of the increment-counting technique, Brown and Jaatoul (1974) found reduced estimates in the EDB and thenar muscles. Rapid decays were also observed in serial assessments. Losses were also found by Daube (1988), who employed a "statistical" method to estimate the number of MUs. Reduced estimates have also been found in the biceps-brachii using the spike-triggered averaging based technique (Brown et al., 1988).

Motor Neuron Disease (MND)

As with ALS, consistently low estimates have been found in MND. In particular, greatly reduced estimates have been found in the EDB (Defaria and Toyonaga, 1978; Hansen and Ballantyne, 1978a; Sica et al., 1974), thenar (Defaria and Toyonaga, 1978; Sica et al., 1974), hypothenar (Carleton and Brown, 1978; Sica et al., 1974) and APL (Defaria and Toyonaga, 1978) muscles. All of these estimates were obtained using a form of the increment-counting technique.

Table A2 Continued: Motor unit estimates for patients with dystrophic disorders

<table>
<thead>
<tr>
<th>Investigator</th>
<th>Muscle</th>
<th>Control</th>
<th>Duchenne</th>
<th>Myotonic</th>
<th>Limb-Girdle</th>
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</thead>
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<tr>
<td>McComas et al. (1978)</td>
<td>hypothenar</td>
<td>386±90</td>
<td>(109)</td>
<td>336±145</td>
<td>(36)</td>
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<td>McComas et al. (1974)</td>
<td>soleus</td>
<td>846±193</td>
<td>(22)</td>
<td>310±44</td>
<td>(7)</td>
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<td>McComas et al. (1977)</td>
<td>soleus</td>
<td>1037±227</td>
<td>(30)</td>
<td>337±203</td>
<td>(31)</td>
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<tr>
<td>McComas et al. (1978)</td>
<td>soleus</td>
<td>957±254</td>
<td>(41)</td>
<td>430±287</td>
<td>(15)</td>
</tr>
</tbody>
</table>
Miscellaneous Neurogenic Disorders

In addition to ALS and MND, MU estimates have been obtained in a number of other disorders. McComas and colleagues (1971c) found reduced estimates in the EDB muscles of patients with Kugelberg-Welander syndrome, hereditary ataxia, idiopathic and carcinomatous polyneuropathies, prolapsed lumbar invertebral discs, peroneal muscular atrophy and old poliomyelitis. Brown and Jaatoul (1974) found slightly reduced estimates in the normal side of hemiplegic patients, while the hemiplegic side showed significant MU reduction. Feasby and Brown (1974) also found a reduced EDB estimate in the affected side of a patient with infantile hemiplegia. Panayiotopoulos and Scarpalezos (1975) found reduced EDB estimates in patients with chronic spinal muscular atrophy.

Sica et al. (1974) found reduced thenar and hypothenar estimates in peroneal muscular atrophy and cervical spondylosis. However, these estimates were not as reduced as those found in MND. By looking at ulnar and median nerve lesions, Sica and colleagues were also able to show how "one population of MUs could be affected while the other was left intact."

Milner-Brown and Brown (1976) found reduced estimates in the EDB, thenar, and hypothenar muscles of patients with various peripheral neuropathies. Reduced estimates have also been found in Guillain-Barre and Porphyric neuropathies (Feasby and Brown, 1974), in diabetic and uraemic neuropathies (Hansen and Ballantyne, 1978,1978a), and in brachial neuropathies (Defaria and Toyonaga, 1978). In addition, patients with unilateral radial nerve palsy presented a reduced number of MUs in the affected side compared with the unaffected APL and other muscles innervated by the median and ulnar nerves (Defaria and Toyonaga, 1978).

Delbeke (1982) used the increment-counting technique to assess grafts to mouth and eyelid muscle groups, and found a greater reduction in the MU numbers of the mouth muscles following grafting.
5. Estimates in Old Age

Extensive studies have been carried out estimating the number of MUs in elderly subjects as compared to younger subjects (Table A5). Significantly reduced MU estimates have been found in the EDB of elderly subjects (Campbell and McComas, 1970, 1973; McComas 1977a). Additionally there is a progressive fall in MU estimates for subjects beyond the age of 60, such that by age 70, the estimate is reduced to half of its original size. Similar results have been seen in the thenar and hypothenar muscle groups (Brown 1972; Sica and McComas, 1971). Again, it is interesting to note that the EDB and thenar muscles are more severely effected than the hypothenar muscles.

Reduced estimates have also been found in the biceps-brachialis using the spike-triggered averaging based technique (Brown et al., 1988). In particular, estimates in subjects 60 years of age or older were approximately half of those of younger subjects.

Using the Macro-EMG based technique, deKoning et al. (1988) found dramatic differences in MU densities for the tibialis anterior in subjects over the age of 60.
<table>
<thead>
<tr>
<th>Investigator</th>
<th>Muscle</th>
<th>Control</th>
<th>ALS</th>
<th>MND</th>
<th>Peripheral Neuropathy</th>
</tr>
</thead>
<tbody>
<tr>
<td>McComas et al. (1971c)</td>
<td>EDB</td>
<td>199±60</td>
<td>14±17</td>
<td></td>
<td>(18)</td>
</tr>
<tr>
<td>Brown and Jaatoul (1974)</td>
<td>EDB</td>
<td>202±58</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milner-Brown and Brown (1976)</td>
<td>EDB</td>
<td>163±84</td>
<td></td>
<td>72±33</td>
<td>(12)</td>
</tr>
<tr>
<td>Hansen and Ballantyne (1978a)</td>
<td>EDB</td>
<td>185±66</td>
<td></td>
<td>72±68</td>
<td>(32)</td>
</tr>
<tr>
<td>Daube (1988)</td>
<td>EDB</td>
<td>185±32</td>
<td>46±32</td>
<td></td>
<td>(15)</td>
</tr>
<tr>
<td>Sica et al. (1974)</td>
<td>thenar</td>
<td>340±87</td>
<td></td>
<td>Decreased</td>
<td>(6)</td>
</tr>
<tr>
<td>Brown and Jaatoul (1974)</td>
<td>thenar</td>
<td>275±75</td>
<td>Decreased</td>
<td></td>
<td>(9)</td>
</tr>
<tr>
<td>Milner-Brown and Brown (1976)</td>
<td>thenar</td>
<td>261±116</td>
<td></td>
<td>127±57</td>
<td>(18)</td>
</tr>
<tr>
<td>Carleton and Brown, (1979)</td>
<td>thenar</td>
<td>261±116</td>
<td></td>
<td>37±83</td>
<td>(6)</td>
</tr>
<tr>
<td>Daube (1988)</td>
<td>thenar</td>
<td>315±48</td>
<td>48±56</td>
<td></td>
<td>(15)</td>
</tr>
<tr>
<td>Sica et al. (1974)</td>
<td>hypothenar</td>
<td>380±79</td>
<td></td>
<td>Decreased</td>
<td>(9)</td>
</tr>
<tr>
<td>Milner-Brown and Brown (1976)</td>
<td>hypothenar</td>
<td>300±125</td>
<td></td>
<td>145±125</td>
<td>(11)</td>
</tr>
<tr>
<td>Carleton and Brown, (1979)</td>
<td>hypothenar</td>
<td>300±125</td>
<td></td>
<td>40±50</td>
<td>(11)</td>
</tr>
</tbody>
</table>

18Numbers in parentheses refer to the number of subjects examined.
<table>
<thead>
<tr>
<th>Investigator</th>
<th>Muscle</th>
<th>Control</th>
<th>Pathology</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>McComas et al. (1971)</td>
<td>EDB</td>
<td>199±60</td>
<td>118±42</td>
<td>Myasthenia Gravis</td>
</tr>
<tr>
<td>McComas et al. (1971c)</td>
<td>EDB</td>
<td>199±60</td>
<td>35±26</td>
<td>Miscellaneous Denervating Disorders</td>
</tr>
<tr>
<td>Ballantyne and Hansen (1974)</td>
<td>EDB</td>
<td>197±49</td>
<td>212±51</td>
<td>Myasthenia Gravis</td>
</tr>
<tr>
<td>Panayiotopoulos and Scarpalezos (1975)</td>
<td>EDB</td>
<td>380±74</td>
<td>64±59</td>
<td>Chronic Spinal Muscular Atrophy</td>
</tr>
<tr>
<td>Hansen and Ballantyne (1977)</td>
<td>EDB</td>
<td>196±54</td>
<td>106±70</td>
<td>Diabetic Neuropathy</td>
</tr>
<tr>
<td>Hansen and Ballantyne (1978a)</td>
<td>EDB</td>
<td>197±49</td>
<td>126±94</td>
<td>Uraemic Neuropathy</td>
</tr>
<tr>
<td>Elder at al. (1983)</td>
<td>EDB</td>
<td>(&gt;100)</td>
<td>54</td>
<td>Infantile Centronuclear Myopathy</td>
</tr>
<tr>
<td>Sica et al. (1974)</td>
<td>thenar</td>
<td>340±87</td>
<td>&lt;150</td>
<td>Peroneal Muscular Atrophy</td>
</tr>
<tr>
<td>Brown and Jaatoul (1974)</td>
<td>thenar</td>
<td>275±75</td>
<td>198±98</td>
<td>Hemiplegia (Normal Side)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>107±45</td>
</tr>
</tbody>
</table>

19Numbers in parentheses refer to the number of subjects examined.
### Table A4 Continued: Motor Unit Estimates for Patients with Miscellaneous Disorders

<table>
<thead>
<tr>
<th>Investigator</th>
<th>Muscle</th>
<th>Control</th>
<th>Pathology</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elder et al. (1983)</td>
<td>thenar</td>
<td>(&gt;150)</td>
<td>77</td>
<td>Infantile Centronuclear Myopathy</td>
</tr>
<tr>
<td>Daube (1988)</td>
<td>thenar</td>
<td>315±48</td>
<td>101±76</td>
<td>Myasthenia Gravis</td>
</tr>
<tr>
<td>Sica et al. (1974)</td>
<td>hypothenar</td>
<td>380±79</td>
<td>&lt;380</td>
<td>Peroneal Muscular Atrophy</td>
</tr>
</tbody>
</table>

#### Table A5: Influence of Age on Motor Unit Estimates

<table>
<thead>
<tr>
<th>Investigator</th>
<th>Muscle</th>
<th>Control (Age&lt;60)</th>
<th>Elderly (Age&gt;60)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Campbell et al. (1973)</td>
<td>EDB</td>
<td>199±60 (41)&lt;sup&gt;20&lt;/sup&gt;</td>
<td>&lt;150</td>
</tr>
<tr>
<td>McComas (1977)</td>
<td>EDB</td>
<td>210±65 (151)</td>
<td>&lt;143</td>
</tr>
<tr>
<td>Barkhaus et al. (1990)</td>
<td>EDB</td>
<td>48±10</td>
<td>23±9</td>
</tr>
<tr>
<td>McComas (1977) thenar</td>
<td>342±97 (115)</td>
<td>&lt;280</td>
<td></td>
</tr>
<tr>
<td>McComas (1977) hypothenar</td>
<td>390±94 (109)</td>
<td>&lt;380</td>
<td></td>
</tr>
<tr>
<td>deKoning (1985)</td>
<td>tibialis Anterior</td>
<td>43±17 (12)</td>
<td>21±9 (9)</td>
</tr>
<tr>
<td>Brown et al. (1988)</td>
<td>biceps-brachialis</td>
<td>911±254</td>
<td>479±220</td>
</tr>
</tbody>
</table>

<sup>20</sup>Numbers in parentheses refer to the number of subjects examined.
6. Inter-Observer Variability

McComas and Sica (1978) state that there is a subjective component associated with the ENS approach which could lead to estimate variations on the order of 10 to 13 percent. This subjective component is associated with the decisions that the observer must make in terms of identifying new MU responses. We evaluated the influence of this subjective component by having three trained observers measure the number of MUs in the muscle of one normal subject (Slawnych et al., 1987). Each observer evaluated the muscle three times, with a two-to-three day period between measurements. In addition, a fourth observer carried out the same measurements using an automated system that performed the decision making process for the observer. We found that the manually obtained estimates varied both between sessions for any given observer, and also between observers. In addition to the subjective component identified by McComas and Sica, random sampling may also contribute to this inter-session variability. (c.f. Representation of the Motor Unit Population). The inter-observer variability, on the other hand, was strictly due to subjective differences between the observers in the manner in which the measurements were carried out. This subjective component could be eliminated through the use of an automated system. Not surprisingly, the estimates obtained using the automated system exhibited a lower coefficient of variation than the manually obtained estimates.

In a similarly study, Brandstater et al. (1989) evaluated the reliability of the original, manual ENS approach by having three trained observers perform the measurements on 20 healthy subjects. Again, each of the observers evaluated the subjects three times, with each measurement being taken on a different day. Brandstater and colleagues concluded that the method, as performed by these trained observers, was not reliable.

The above studies underscore the need for automating the measurements associated with ENS approach. Not only would this eliminate the subjective component associated with the
measurement, but it would also expedite the measurement and facilitate the comparison of results obtained from different laboratories.

7. Comparison with Other Approaches

McComas and Sica (1978) evaluated the utility of using MU estimates to assess muscle status by comparing estimates obtained using the original increment counting technique with a quantitative EMG approach in which the following parameters were extracted: the mean number of phases and the durations of MUAPs recruited at minimal force isometric contractions, and the mean amplitudes and densities of the interference pattern elicited at maximal force isometric contractions. Both the EDB and abductor pollicis brevis (APB) muscles were examined in subjects experiencing the following neuromuscular disorders: L5 root lesion, peripheral neuropathy, motoneuron disease, and aging. The results, which are given in Table A6, show that when the motor unit estimates are employed, 49 of 53 muscles as classified as being neurogenic. The quantitative EMG parameters, on the other hand, only classified 29 of these muscles as being neurogenic. In order for a muscle to be classified as being neurogenic, only one of the four parameters had to be outside of the normal range. It should be pointed out that for the quantitative EMG classification, values were regarded as being abnormal only if they differed by more than two standard deviations from the corresponding control mean. For the MU estimates, on the other hand, estimates were regarded as abnormal if they were less than the lower limit of the normal group. The two standard deviation range was not employed since the distribution of the control estimates was skewed. However, even if the normal range was extended to two standard deviations, 45 of the 53 muscles would still be classified as being neurogenic.

No correlation was found between the reduction in the MU estimates and the degree of abnormality in any of the four quantitative EMG parameters. Interestingly, in four of the patients with suspected lumbosacral root pathology, the motor unit estimates were found to be normal but
at least one of the quantitative EMG parameters were found to be abnormal. On the other hand, all of the quantitative EMG parameters were found to be normal in three of the EDB muscles while the MU estimates were reduced. In fact, it was estimated that one of the muscles contained have only 16 MUs.

Table A6: Comparison of Quantitative EMG and MU Estimate Measures

<table>
<thead>
<tr>
<th>Muscle Examined</th>
<th>Number of Muscles Classified as Neurogenic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MUE Analysis</td>
</tr>
<tr>
<td>EDB (23)</td>
<td>20</td>
</tr>
<tr>
<td>APB (30)</td>
<td>29</td>
</tr>
<tr>
<td>Total (53)</td>
<td>49</td>
</tr>
</tbody>
</table>

Daube (1990) also carried out a comparative study in which MU estimates obtained from the serial assessment of ten amyotrophic lateral sclerosis (ALS) patients were compared with the amplitudes and densities of interference patterns elicited at maximal force isometric contractions. The motor unit estimates were calculated in three different ways: 1) using the original ENS approach, 2) using the statistical approach developed by Daube, and 3) using the VMA approach based on the semi-quantitative analysis of MUs recorded with needle electrodes. Each of the three methods yielded estimates that correlated well with one another. However, the correlation between the estimates and the quantitative EMG measures was not as good.

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21Numbers in parentheses refer to the number of muscles examined.
APPENDIX 3. ANATOMICAL VALIDATION

The validity of the motor unit estimation schemes can be tested by counting the number of motor axons supplying muscles in which electrophysiological testing has been performed. Such studies have been carried out on both animals and humans.

1. Animal Studies

Eisen et al. (1974) found good correlation between physiological and anatomical estimates in the rat soleus muscle. In particular, reduced MU numbers were found in neurogenic disorders in which reinnervation has taken place, while normal numbers were found in ischaemic myopathy. The physiological estimates were obtained by employing the method of McComas et al. (1971a), whereas the anatomical estimates were obtained by taking 50% of the number of fibers with diameters (>5.5 µm). However, it should be noted that the nerve and muscle were both exposed for the electrophysiological assessment.

Law and Caccia (1975) similarly found good correlation between electrophysiological and anatomical estimates for the soleus muscles of normal and dystrophic mice. Again, the nerve and muscle were exposed for the electrophysiological examination. Investigating the soleus and plantaris muscles of dystrophic mice, Montgomery and Swenarchuk (1978) found reduced numbers of myelinated fibers innervating these muscles. While a parallel electrophysiological study was not carried out, the results tend to support the general findings of reduced MU numbers in dystrophic muscles. Habgood et al. (1976) used the increment-counting technique on an in vivo nerve/muscle preparation to investigate the mouse soleus muscle. Their findings correlate well with the anatomical estimates of Law and Caccia (1975).

Peyronnard and Lamarre (1977) compared anatomical and electrophysiological estimates in normal and deafferentated EDB muscles of monkeys. Deafferentation eliminated the difficulty of
distinguishing between the sensory and motor components of an intact nerve. They found acceptable correspondence between the values in 5 animals. However, in a sixth animal in which a ventral root had been damaged during the deafferentation procedure, electrophysiological estimates were up to two times higher than the anatomical counts. These higher estimates, which were attributed to irregular neuromuscular transmission at the newly reinnervated muscle fibers, raise doubts about the reliability of the technique in partial reinnervation.

Wray (1969) reports anatomical estimates of 220 MUs for the APB in the baboon. Since the APB is one of the three muscles making up the thenar muscle group and assuming that each of the other muscles consists of the same number of MUs, the thenar MU estimate would be on the order of 660. This is above the electrophysiological range normally found in humans. However, on the basis of a comparison with human anatomical estimates obtained by Feinstein et al (1955), Wray believes that there may be a genuine species difference between man and baboon.

2. Human Studies

Feinstein et al. (1955) were one of the first groups to provide anatomical data for the numbers and sizes of human motor units. Using their value of 119 MUs for the 1st dorsal interosseus as being an average value for the small muscles of the hand, thenar and hypothenar estimates should be on the order of 360. This falls well within the range of estimates obtained electrophysiologically.

McComas and colleagues (1971a) examined two peroneal nerve specimens and found the anatomical estimates to be within the upper electrophysiological range. Lee et al. (1975) found good correspondence between electrophysiological and anatomical estimates for the thenar muscle group.
Tomlinson and Irving (1974) confirmed the electrophysiological finding of decreased motor unit numbers in elderly patients by counting cell nuclei in serial lumbosacral sections. Bolton et al. (1979) carried out a parallel electrophysiological/anatomical study of the EDB muscle in subjects with subacute or chronic sensorimotor polyneuropathy. However, only the density of the large myelinated fibers per square millimeter, not the actual number, was calculated. Nevertheless, a high correlation was found between the density of large myelinated fibers (> 4.6 μm) and the MU estimate. Interestingly, they noted that in the disorders they studied, the amplitude of the compound action potential was as accurate an estimate of the large fiber density as was the MU estimate.

Carvalho and colleagues (1988) have recently estimated the number of MUs in the opponens digiti minimi (one of the hypothenar muscles) to be 158. Assuming that the remaining hypothenar muscles have an equal number of MUs, the estimated number of MUs for the group would be on the order of 480. This is in the high normal range of electrophysiological estimates. However, Carvalho et al. assumed that 60% of the large myelinated fibers were alpha motor. By changing this estimate to 50%, the motor unit estimate would be on the order of 400, well within electrophysiological ranges.

3. Problems Associated with Anatomical Estimation Methods
As with their electrophysiological counterpart, anatomical estimates are also prone to errors. In particular, in the majority of experiments in which deafferentation was not performed, the relative proportion of sensory and motor axons must be estimated. In addition, the motor component must be further subdivided into alpha and non-alpha components. Estimates for the motor component range between 40 and 60% (Boyd, 1968). On the other hand, if deafferentation is performed, care must be taken to prevent damage to ventral roots (Peyronnard and Lamarre, 1977).
Additionally, axonal branching proximal to the area of investigation would corrupt the count, as would motor axons innervating muscles other than the one(s) being investigated.

Finally, if some fibers were experiencing proximal conduction block (e.g. due to segmental demyelination), these fibers would be included in the anatomical count but would not contribute to the electrophysiological estimate.
APPENDIX 4. DECOMPOSING FULL RESPONSE SETS INTO THEIR CONSTITUENT MUAPs

When two responses are observed (i.e. n=1 where n is the number of probabilistically active MUs), the constituent MUAP is simply obtained by subtracting the smallest response from the largest response.

When n=2, there are two ways in which each MUAP can be extracted. In particular, they can be obtained by subtracting the smallest response from the 2nd and 3rd smallest responses, or by subtracting the 2nd and 3rd smallest responses from the 4th smallest (i.e. the largest) response. This can be best appreciated by looking at Table A7, where the responses (and their constituent MUs) are explicitly listed.

<table>
<thead>
<tr>
<th>Response</th>
<th>Status of MU₁</th>
<th>Status of MU₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>R₁</td>
<td>inactive</td>
<td>inactive</td>
</tr>
<tr>
<td>(Smallest)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R₂</td>
<td>active</td>
<td>inactive</td>
</tr>
<tr>
<td>(2nd smallest)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R₃</td>
<td>inactive</td>
<td>active</td>
</tr>
<tr>
<td>(3rd smallest)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R₄</td>
<td>active</td>
<td>active</td>
</tr>
<tr>
<td>(4th smallest)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Similarly, when eight responses are observed, there are several ways in which each of the three MUAPs can be extracted. Specifically, the two smallest MUAPs can be obtained by subtracting the smallest response from the second and third smallest responses or by subtracting the 6th and 7th smallest responses from the 8th smallest response. The extraction of the third MUAP is
somewhat more complicated. If the response generated by subtracting the smallest response from the fourth smallest response is not equal to the combined response of the first two MUAPs, then this subtracted signal represents the third MUAP. Otherwise, the third MUAP is given by the difference between the fifth smallest response and the smallest response (c.f. Table A8).

Table A8: Interpreting the eight responses in terms of their constituent MUs.

<table>
<thead>
<tr>
<th>Response</th>
<th>Interpretation if $R_4 - R_1 \neq MU_1 + MU_2$</th>
<th>Interpretation if $R_4 - R_1 = MU_1 + MU_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Status of $MU_1$</td>
<td>Status of $MU_2$</td>
</tr>
<tr>
<td>$R_1$</td>
<td>inactive</td>
<td>inactive</td>
</tr>
<tr>
<td>(Smallest)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$R_2$</td>
<td>active</td>
<td>inactive</td>
</tr>
<tr>
<td>(2nd smallest)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$R_3$</td>
<td>inactive</td>
<td>active</td>
</tr>
<tr>
<td>(3rd smallest)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$R_4$</td>
<td>inactive</td>
<td>inactive</td>
</tr>
<tr>
<td>(4th smallest)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$R_5$</td>
<td>active</td>
<td>active</td>
</tr>
<tr>
<td>(5th smallest)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$R_6$</td>
<td>active</td>
<td>inactive</td>
</tr>
<tr>
<td>(6th smallest)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$R_7$</td>
<td>inactive</td>
<td>active</td>
</tr>
<tr>
<td>(7th smallest)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$R_8$</td>
<td>inactive</td>
<td>active</td>
</tr>
<tr>
<td>(8th smallest)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Under certain circumstances, it may be difficult to rank the order of the responses. An example of such a situation is one in which the responses are highly polyphasic. In this case, we can extract what we have termed candidate MUAPs by looking at the differences between all pairs of responses. We can look at all of the possible groupings of n candidate MUAPs (where n is the number of MUs we are extracting from the observed responses) and generate full response sets for each one. The constituent MUs are obtained from the grouping which yields the response set that matches the actual response set.
APPENDIX 5. EXAMINATION OF THE CASE IN WHICH 3 MUs ARE PROBABILISTICALLY ACTIVATED

The observation-rate based decomposition algorithm that we developed in Section 3.4 requires that the two most prominent responses be clearly identified from the remaining responses. The most prevalent response can be clearly identified in cases in which none of the MUs are associated with an activation probability of 0.5. The 2nd most prevalent response can also be clearly identified if all of the MUs are associated with different activation probabilities. However, if two or three of the MUs have identical activation probabilities, then a number of responses will have the same, 2nd highest observation probability. Fortunately, the difference between any of these responses and the most prominent response yields a MUAP (c.f. Table A9).

In cases in which one of the MUs is associated with an activation probability of 0.5, there is no single response that is most prominent. Rather, there are two responses with equally high observation probabilities. The MUAP can be simply obtained by evaluating the difference between these two responses.

If two MUs are associated with activation probabilities of 0.5, then there will be four responses with equally high observation probabilities. Unfortunately, the differences between all possible pairs of these responses do not always yield a MUAP. Hence, in these cases, we do not extract any MUAPs.

Finally, if all three MUs are associated with activation probabilities of 0.5, then each of the eight possible responses will be associated with an identical observation probability. However, this does not pose a problem as there is a good probability that the full response set will be observed.
Table A9: Evaluation of the probability space for the case in which 3 MUs are undergoing probabilistic activation.

<table>
<thead>
<tr>
<th>Probability of Activating Each MU</th>
<th>Probability of Observing Each Response</th>
<th>Extraction of MUs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Probability of Activating MUs</td>
<td>R₁, R₂, R₃, R₄, R₅, R₆, R₇, R₈</td>
<td></td>
</tr>
<tr>
<td>MU₁, MU₂, MU₃</td>
<td>(0,0,0), (0,1,0), (0,0,1), (1,1,0), (1,0,1), (0,1,1), (1,1,1)</td>
<td></td>
</tr>
<tr>
<td>0.1 0.1 0.1 0.729 0.081 0.081 0.081 0.009 0.009 0.009</td>
<td>MU₁ = R₂, R₃, R₄, R₅, R₆, R₇, R₈</td>
<td>MU₂ = R₃, R₁, R₄, R₅, R₆, R₇, R₈</td>
</tr>
<tr>
<td>0.1 0.1 0.2 0.648 0.072 0.072 0.072 0.008 0.18 0.018</td>
<td>MU₂ = R₃, R₁, R₄, R₅, R₆, R₇, R₈</td>
<td>MU₃ = R₄, R₁, R₅, R₆, R₇, R₈</td>
</tr>
<tr>
<td>0.1 0.1 0.3 0.567 0.063 0.063 0.243 0.007 0.027 0.027</td>
<td>MU₃ = R₄, R₁, R₅, R₆, R₇, R₈</td>
<td>MU₄ = R₅, R₁, R₆, R₇, R₈</td>
</tr>
<tr>
<td>0.1 0.1 0.4 0.486 0.054 0.054 0.324 0.006 0.036 0.036</td>
<td>MU₄ = R₅, R₁, R₆, R₇, R₈</td>
<td>MU₅ = R₆, R₁, R₇, R₈</td>
</tr>
<tr>
<td>0.1 0.1 0.5 0.405 0.045 0.045 0.405 0.005 0.045 0.045</td>
<td>MU₅ = R₆, R₁, R₇, R₈</td>
<td>MU₆ = R₇, R₈</td>
</tr>
<tr>
<td>0.1 0.2 0.1 0.648 0.072 0.072 0.072 0.008 0.018 0.018</td>
<td>MU₂ = R₃, R₁, R₄, R₅, R₆, R₇, R₈</td>
<td>MU₃ = R₄, R₁, R₅, R₆, R₇, R₈</td>
</tr>
<tr>
<td>0.1 0.2 0.2 0.576 0.064 0.144 0.144 0.016 0.016 0.016</td>
<td>MU₂ = R₃, R₁, R₄, R₅, R₆, R₇, R₈</td>
<td>MU₃ = R₄, R₁, R₅, R₆, R₇, R₈</td>
</tr>
<tr>
<td>0.1 0.2 0.3 0.504 0.056 0.126 0.014 0.014 0.014 0.014</td>
<td>MU₂ = R₃, R₁, R₄, R₅, R₆, R₇, R₈</td>
<td>MU₃ = R₄, R₁, R₅, R₆, R₇, R₈</td>
</tr>
<tr>
<td>0.1 0.2 0.4 0.432 0.048 0.108 0.288 0.012 0.032 0.012</td>
<td>MU₂ = R₃, R₁, R₄, R₅, R₆, R₇, R₈</td>
<td>MU₃ = R₄, R₁, R₅, R₆, R₇, R₈</td>
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<tr>
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<td>MU₂ = R₃, R₁, R₄, R₅, R₆, R₇, R₈</td>
<td>MU₃ = R₄, R₁, R₅, R₆, R₇, R₈</td>
</tr>
<tr>
<td>0.1 0.3 0.1 0.567 0.063 0.243 0.063 0.027 0.027 0.027</td>
<td>MU₃ = R₄, R₁, R₅, R₆, R₇, R₈</td>
<td>MU₄ = R₅, R₁, R₆, R₇, R₈</td>
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<tr>
<td>0.1 0.3 0.2 0.504 0.056 0.216 0.126 0.024 0.014 0.014</td>
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<td>MU₅ = R₆, R₁, R₇, R₈</td>
</tr>
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<td>MU₆ = R₇, R₈</td>
</tr>
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<td>MU₇ = R₈</td>
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<tr>
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<td>MU₂ = R₃, R₁, R₄, R₅, R₆, R₇, R₈</td>
<td>MU₃ = R₄, R₁, R₅, R₆, R₇, R₈</td>
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<tr>
<td>0.1 0.4 0.2 0.432 0.048 0.288 0.108 0.032 0.012 0.072</td>
<td>MU₂ = R₃, R₁, R₄, R₅, R₆, R₇, R₈</td>
<td>MU₃ = R₄, R₁, R₅, R₆, R₇, R₈</td>
</tr>
</tbody>
</table>

*the numbers enclosed by the parentheses refer to the status of MU₁, MU₂, and MU₃, respectively, with "0" indicating that the MU is inactive and "1" indicating that the MU is active.*
APPENDIX 6. SPECIFICATION OF THE THRESHOLD LEVEL

As stated in Chapter 4, classification schemes usually begin by extracting features from the recorded responses. For the purposes of the following discussion, let us designate the transformation from the observation space to the feature space as \( H(x(t)) \) (a summary of the definitions for all of the terms employed in this section can be found in Table A10). Hence the Euclidean distance between any two signal classes \( a_n(t) \) and \( a_m(t) \) based on the feature space is given by

\[
d_{nm} = \left( \sum_{j=1}^{k} (h_j(a_m(t)) - h_j(a_n(t)))^2 \right)^{1/2}
\]  

where \( h_j(a_n(t)) \) is the \( j \)th feature for the signal and \( k \) is the dimension of the feature space. We must now specify the amount of separation needed to resolve the different responses. To begin, let us specify the probability of correctly attributing potentials from the \( i \)th unit to the \( i \)th unit as \( 1 - \alpha_i \). Assuming that the noise (calculated in terms of the feature space) is normally distributed with unit standard deviation, we can establish the radius \( R \) required to guarantee that potentials from unit \( i \) are correctly assigned with a probability \( 1 - \alpha_i \). Choosing a set of coordinates centered on \( H(a_i(t)) \), the distribution of \( H(x(t)) \) when \( x(t) \) is an observation of unit \( a_i(t) \) is given by

\[
p(H(x(t))) = \frac{1}{(2\pi)^{k/2}} \prod_{i=1}^{k} \exp\left(\frac{-h_i^2}{2}\right)
\]

where \( h_i \) is the \( i \)th element of \( H \) (Heetderks, 1978). We then integrate \( p(H(x(t))) \) over a radially symmetric region to find a value of \( R \) such that the integral equals \( 1 - \alpha_i \), yielding

...
Let us first consider the case in which $K=1$. A typical example of this is in one in which responses are classified on the basis of their amplitudes. In particular, we have to solve the following

$$1-a_i = \int \cdots \int_{(h_1^2 + h_2^2 + \ldots + h_n^2) < R^2} \left( \frac{1}{(2\pi)^{k/2}} \prod_{i=1}^{k} \exp(-\frac{x_i^2}{2}) \right) dx_i$$

For a correct classification rate of 95%, we find that $R=1.96$. If we increase the rate to 99%, $R$ increases to 2.50.

In general, the solution to equation 4 can be specified in terms of a Chi-squared distribution

$$1-\alpha_i = \chi^2(R^2, k)$$

where $\chi^2(R^2, k)$ is the cumulative Chi-squared distribution from 0 to $R^2$ with $k$ degrees of freedom (Heetderks, 1978). The results for various dimensions and significance levels are given in Table A11.
<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
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<tr>
<td>$X(t)$</td>
<td>the observed signal sequence</td>
</tr>
<tr>
<td>$A(t)$</td>
<td>the unique responses that make up the observed signal sequence</td>
</tr>
<tr>
<td>$x_j(t)\ 1 \leq j \leq N_{obs}$</td>
<td>the $j$th element of the observed signal sequence</td>
</tr>
<tr>
<td>$a_j(t)\ 1 \leq j \leq N_{unique}$</td>
<td>the $j$th element of the unique signal sequence</td>
</tr>
<tr>
<td>$N_{obs}$</td>
<td>the number of observed responses</td>
</tr>
<tr>
<td>$N_{unique}$</td>
<td>the number of unique responses</td>
</tr>
<tr>
<td>$\epsilon$</td>
<td>background noise (assumed to be Gaussian in nature)</td>
</tr>
<tr>
<td>$d_{ij}$</td>
<td>distance between the $i$th and $j$th responses</td>
</tr>
<tr>
<td>$H(x_j(t))$</td>
<td>representation of $x_j(t)$ in terms of its feature space</td>
</tr>
<tr>
<td>$k$</td>
<td>Dimension of the feature space</td>
</tr>
<tr>
<td>$1-\alpha_i$</td>
<td>the probability of correctly detecting a signal from class $i$</td>
</tr>
<tr>
<td>$\beta_{ij}$</td>
<td>the probability of mistaking a signal from class $j$ for a signal from class $i$</td>
</tr>
<tr>
<td>$p(x)$</td>
<td>the probability density function of $x$</td>
</tr>
<tr>
<td>$R$</td>
<td>acceptance radius for a signal for a specified value of $1-\alpha_i$</td>
</tr>
<tr>
<td>$D$</td>
<td>the minimum required separation between responses for specified values of $1-\alpha_i$ and $\beta_{ij}$</td>
</tr>
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</table>
Table A11: Acceptance radius as a function of classifier dimension and confidence interval

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<tr>
<th>Dimension</th>
<th>$1-\alpha_i = 0.80$</th>
<th>$1-\alpha_i = 0.90$</th>
<th>$1-\alpha_i = 0.95$</th>
<th>$1-\alpha_i = 0.99$</th>
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<td>6.34</td>
<td>6.62</td>
<td>7.13</td>
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In addition to the correct classification rate, we also have to state the false alarm probability $\beta_i$, which is the probability of incorrectly attributing responses from other units to the $i$th unit. For example, let us consider the case in which the dimension of the feature space is 1 and the confidence interval is 95%. Thus we are effectively missing 5% of the occurrences of this signal with 2.5% occurring on either end of the distribution (Figure A2a). Hence if we separate two responses by a distance of twice the acceptance radius, we can expect that 2.5% of the responses from each class will be misclassified (Figure A2b).

In general, the minimum required distance between signal classes must satisfy the following (Heetderks, 1978)

$$b_{ij} = \left( \frac{1}{\sqrt{2\pi}} \int_{-R}^{R} \exp\left(\frac{(x-D)^2}{2}\right) dx \right)^k$$

where $k$ represents the dimension of the feature space, $R$ represents the previously calculated acceptance radius, and $D$ is the minimum separation value. Equation 6 was solved using a numerical integration technique for various values of $1-\alpha_i$ (and hence $R$) and $\beta_i$. The results can be found in Table A12.
Figure A2: Evaluating the influence of inter-signal separation on the false alarm rate. a) The probability density function of a particular signal represented in terms of 1 feature. b) By setting the inter-signal distance to 2R, we are effectively setting the false alarm rate to 2.5%.
Table A12: Minimum signal class separation as a function of classifier dimension and confidence intervals.

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<th>Dimension</th>
<th>Minimum Inter-Signal Separation</th>
<th>( \beta_i = 0.20 )</th>
<th>( \beta_i = 0.10 )</th>
<th>( \beta_i = 0.05 )</th>
<th>( \beta_i = 0.01 )</th>
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APPENDIX 7.  SUMMARIES OF THE DATA ACQUISITION AND ANALYSIS PROCEDURES

Data Acquisition

Data acquisition begins with the recording of two maximal muscle responses. These are obtained by supra-maximally stimulating the nerve to the muscle. That is, the stimulus intensity is gradually increased until there is no observable increase in the size of the evoked muscle response. The responses are then saved to a file. In order to keep the size of this file as small as possible, the responses are stored in integer format (specifically the values recorded by the A/D converter along with the gain value at which they are recorded).

Four noise epochs are then recorded with the stimulator turned off, and the responses are saved to a file. This is followed by the determination of the threshold stimulation level at which MUAPs are first observed. The nerve is repetitively stimulated 50 times at this stimulus level, and the associated response sequence is recorded. At this point, the operator has the option of either saving the sequence to a file or discarding it. A typical reason for discarding the sequence would be the presence of excessive background voluntary muscle activity in the recordings. The process of selecting a stimulus intensity, stimulating the nerve, and either saving or discarding the response sequence is repeated a number of times such that a representative MU sample can be obtained. The criteria for the selection of these new stimulus settings are:

1) the presence of multiple responses (thus indicating that one or more MUs are undergoing probabilistic activation), and

2) that the sequence does not include many responses recorded in previous sequences.

The entire procedure is summarized in Figure A3.

Data Analysis

Data analysis begins with the retrieval of the data file and the normalization of the data values by their respective gain settings. The average M-Wave amplitude and area are then calculated. The
M-Waves are also used to establish the time window with which to analyze the remaining compound responses. Noise statistics are then obtained, and if 60 Hz. activity is detected, a noise template is constructed using the pre- and post-evoked potential segments and subtracted from the evoked potential segment. Each response sequence is then reduced to its constituent MUAP(s) as follows:

1. The first ten responses are discarded, and the pre- and post-evoked potential segments of the remaining responses are examined for the presence of voluntary muscle activity. If activity is found in these segments, the response is discarded.

2. The remaining responses are reduced to their unique response set using the nearest neighbor clustering technique discussed in Section 4.4. In performing this classification, the threshold levels are set such that the correct classification and false alarm rates were 95% and 5%, respectively (cf. Section 4.3).

3. The constituent MU(s) are then extracted using the observation-rate-based decomposition protocol described in Section 3.4.

The extracted MUAPs are then averaged to yield the mean MUAP. The amplitude and area of this response are then calculated and divided into the amplitude and area of the M-Wave response, yielding the amplitude and area based estimates, respectively. Figure A4 summarizes the entire analysis procedure.
Get patient Information (name, gender, age ...) and save to file

Specify Acquisition Parameters (number of Stimuli per train, stimulation frequency, stimulus pulse duration)

Acquire two M-Wave responses by supra-maximally stimulating the nerve innervating the muscle

Save the M-Wave data (saved in integer format along with gain setting)

Acquire four noise responses (accomplished by setting the stimulus intensity to 0)

Save the Noise data (saved in integer format along with gain setting)

Acquire a response sequence by repetitively stimulating the nerve

Figure A3: Flowchart summary of the data acquisition procedure.
Begin
Read data file (stored in integer format)

Normalize data by their gain settings (therefore converted to floating point)

Classify remaining responses into their unique responses using the nearest-neighbour clustering procedure (§4.4)

Calculate the amplitude and area of the M-Wave response

Calculate the average RMS noise level of the four noise epochs and check for presence of 60 Hz.

J = 1

Decompose sequence into its constituent MUAP(s) (readily done since full response sequence is present - Appendix IV)

Extract a MUAP by evaluating the difference between the two most prominent responses (§3.4)

Remove 60 Hz by constructing a template using pre and post segments of the response epochs (§5.3)

Remove the first 10 responses and check remaining responses for excessive noise

Number of unique responses = 2, 4 or 8?

Yes

Decompose sequence into its constituent MUAP(s) (readily done since full response sequence is present - Appendix IV)

No

J = J + 1

Number of unique responses >1 and <8?

Yes

Extract a MUAP by evaluating the difference between the two most prominent responses (§3.4)

J = J + 1

No

60 Hz. Present?

Yes

Remove 60 Hz by constructing a template using pre and post segments of the response epochs (§5.3)

No

J = J + 1

Calculate mean MUAP by averaging the extracted MUAPs

Calculate the motor unit estimate

Exit

Figure A4: Flowchart summary of the data analysis procedure.
APPENDIX 8. DERIVATIONS OF $D_{\text{min}}$ and $f_s$

1. Calculating the Minimum Required Distance to Uniquely Observe Each MUAP

In order to observe each MUAP isolated in time from the remaining MUAPs, we require that the latency difference between all MUAP pairs be larger than the maximum MUAP duration ($t_{\text{max}}$). Hence, we require that

$$|t_i - t_j| \geq t_{\text{max}}$$  \[
1
\]

for all possible values of $i$ and $j$. Knowing the maximum motor neuron conduction velocity ($V_{\text{max}}$), and the minimum difference in conduction velocity values between any two motor neurons ($\Delta V_{\text{min}}$), the above can be satisfied by the following condition

$$\frac{D_{\text{min}}}{V_{\text{max}} - \Delta V_{\text{min}}} - \frac{D_{\text{min}}}{V_{\text{max}}} \geq t_{\text{max}}$$ \[
2
\]

where $\frac{D_{\text{min}}}{V_{\text{max}}}$ and $\frac{D_{\text{min}}}{V_{\text{max}} - \Delta V_{\text{min}}}$ are the latencies of the fastest and second fastest responses, respectively. Therefore, we have that

$$D_{\text{min}} \geq \frac{t_{\text{max}} V_{\text{max}}}{\Delta V_{\text{min}}} (V_{\text{max}} - \Delta V_{\text{min}})$$ \[
3
\]
2. Calculating the Minimum Required Sampling Frequency

The minimum frequency required to detect the contribution of each MUAP to \( h_s(t) \) can be derived in a similar fashion to \( D_{\text{min}} \). In this case, we require that

\[
|t_i - t_j| \geq t_s
\]  \hspace{1cm} [4]

where \( t_s \) is the time increment between successive samples. Given that \( f_s \) is the sampling frequency, we have that \( t_s = 1/f_s \). Hence, equation 4 can also be expressed as follows:

\[
|t_i - t_j| \geq \frac{1}{f_s}
\]  \hspace{1cm} [5]

As with the calculation of \( D_{\text{min}} \), equation 5 can be satisfied by considering the two fastest motor neurons. Hence we have that

\[
\frac{D}{V_{\text{max}} - \Delta V_{\text{min}}} - \frac{D}{V_{\text{max}}} \geq \frac{1}{f_s}
\]  \hspace{1cm} [6]

where \( V_{\text{max}} \) is the maximum motor conduction velocity, \( \Delta V_{\text{min}} \) is the minimum difference in conduction velocity values between any two MUs, and \( D \) is the distance between the two stimulation sites. Thus we have that

\[
f_s \geq \frac{D \Delta V_{\text{min}}}{(V - \Delta V_{\text{min}}) V}
\]  \hspace{1cm} [7]

In order to facilitate the detection of each delta function, we would like to have at least one sample between consecutive delta functions. Hence we have that

\[
f_s \geq \frac{2 D \Delta V_{\text{min}}}{(V - \Delta V_{\text{min}}) V}
\]  \hspace{1cm} [8]
APPENDIX 9. EFFECT OF NON-ZERO DISTANCE VALUES ON 
THE SYSTEM RESPONSE FUNCTION

Let us consider the simple case in which we have two MUs. The M-Wave potentials that would 
be observed at distances $d_1$ and $d_2$ are given by

$$ mw_1(t) = w_1 a(t - \frac{d_1}{v_1}) + w_2 a(t - \frac{d_1}{v_2}) $$  \[1\]

and

$$ mw_2(t) = w_1 a(t - \frac{d_2}{v_1}) + w_2 a(t - \frac{d_2}{v_2}) $$ \[2\]

respectively. Hence we have that

$$ H(f) = \frac{w_1 e^{-j2\pi df/d_1 v_1} + w_2 e^{-j2\pi df/d_2 v_2}}{w_1 e^{-j2\pi df/d_1 v_1} + w_2 e^{-j2\pi df/d_2 v_2}} $$ \[3\]

Equation 3 can be re-written as follows

$$ H(f) = e^{j2\pi f(d_2 - d_1)/v_1} \left( \frac{w_1 e^{-j2\pi df/(v_2 - v_1)}/v_1 v_2}{w_1 e^{-j2\pi df/(v_2 - v_1)}/v_1 v_2 + w_2} \right) $$ \[4\]

$$ + e^{j2\pi f(d_2 - d_1)/v_2} \left( \frac{w_2 e^{-j2\pi df/(v_2 - v_1)}/v_1 v_2}{w_1 e^{-j2\pi df/(v_2 - v_1)}/v_1 v_2 + w_2} \right) $$

Assuming that $v_2$ is larger than $v_1$, a series expansion can be used to express the above equation 
as follows

$$ H(f) = e^{j2\pi f(d_2 - d_1)/v_1} e^{-j2\pi df/(v_2 - v_1)}/v_1 v_2 \frac{w_1}{w_2} (1 - B + B^2 - B^3 + ...) $$ \[5\]

$$ + e^{j2\pi f(d_2 - d_1)/v_2} (1 - B + B^2 - B^3 + ...) $$

where
Equation 5 can also be expressed as follows:

\[ B = \frac{w_1}{w_2} e^{j2\pi f d_1 (v_2 - v_1)} / v_1 v_2 \]  \[6\]

Taking the inverse Fourier transform of the above yields

\[ H(f) = \sum_{k=0}^{\infty} e^{j2\pi f (d_2 - d_1) / v_1} (-1)^k \left( \frac{w_1}{w_2} \right)^{k+1} e^{j2\pi f d_1 (v_2 - v_1) / v_1 v_2} \]

\[ + \sum_{k=0}^{\infty} e^{j2\pi f (d_2 - d_1) / v_2} (-1)^k \left( \frac{w_1}{w_2} \right)^k e^{j2\pi f d_1 (v_2 - v_1) / v_1 v_2} \]  \[7\]

Taking the inverse Fourier transform of the above yields

\[ h(t) = \sum_{k=0}^{\infty} (-1)^k \left( \frac{w_1}{w_2} \right)^{k+1} d(t - t_1(k)) + \sum_{k=0}^{\infty} (-1)^k \left( \frac{w_1}{w_2} \right)^k d(t - t_2(k)) \]  \[8\]

where

\[ t_1(k) = \frac{d_2 - d_1}{v_1} + \frac{(k+1) d_1 (v_2 - v_1)}{v_1 v_2} \]  \[9\]

and

\[ t_2(k) = \frac{d_2 - d_1}{v_2} + \frac{k d_1 (v_2 - v_1)}{v_1 v_2} \]  \[10\]

As we can see, \( h(t) \) no longer consists of just two spikes located at the appropriate time delays, but rather of a number of spikes located at different latencies. Hence, the system is not linear.