AUTOMATIC ESTIMATION OF THE NUMBER OF MUSCLE MOTOR UNITS

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ABSTRACT

A manual method of estimating the number of functional motor units within a selected muscle has been implemented as an automatic system. The system has been developed in FORTRAN 77 on a PDP-11/34 minicomputer. This system employs closed loop control of the stimulator, thus eliminating any active role of the operator during the testing. The heuristic decision-making processes required of the operator in the manual method have been analyzed and replaced with a defined algorithm incorporating quantifiable decision criteria. The automated motor unit counting system has been demonstrated using a number of human subjects. The system is suitable for use in a research clinical environment, providing a flexible tool for clinical trials or for further development of the technique.

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TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

LIST OF TABLES

CHAPTER 1.

INTRODUCTION

1.1. The Work in Perspective

Computer technology has found diverse applications in the medical sciences - the computer's ability to quickly collect and reduce large amounts of data has been exploited in the areas of patient assessment and medical research. The work described here has been to automate an existing technique which has been applied to both of these areas. This chapter provides an introduction to the concepts and methods used throughout the thesis. In particular, the motor unit is defined and evoked potential electromyography is discussed. The method developed by McComas (1971a) to estimate the number of motor units is presented, followed by the objectives and scope of this thesis.

1.1.1. Definition and Overview of the Motor Unit

The motor unit (MU) is the smallest addressable element of force generation within a skeletal muscle. The MU consists of a motor neuron (MN), and all of the muscle fibers which are innervated by that MN. A healthy human muscle contains many MUs, each of which contributes to the total tension produced by the muscle. The motor control system selec-

tively activates the MUs comprising the various muscles to achieve coordinated torque and movement at the joints.

To initiate the contraction of the muscle fibers of a MU, an electrochemical event, the action potential (AP), propagates along the MN towards the muscle fibers. The AP is a transient all-or-nothing phenomenon which evokes a brief contraction of the muscle fibers, a twitch, which lasts from 10 to 100 mS (Guyton, 1982). The strength of the MU contraction can be increased by evoking a series of twitches at a rate such that successive contractions begin before the previous ones are complete, resulting in a summation effect. The tension of the whole muscle is moderated by a combination of two mechanisms: increasing or decreasing the number of MUs recruited, and altering the frequency of excitation of the MUs to modulate the tension produced by the individual MUs.

The number of MUs normally found in a muscle is related to the control refinement required of the muscle. The average size of the MUs, that is, the number of muscle fibers per MN, is related to the force required of the muscle. Thus the platysma, a muscle controlling the position of the jaw, is relatively small (27,000 muscle fibers) and has many (1000) MUs, whereas a large (1,000,000 fibers) calf muscle, the medial head of the gastrocnemius, may have only 580 MUs, but with approximately 70 times as many fibers per MN (Feinstein, 1955).

Two qualities of motor control can be reduced should some of the MUs in a muscle become inoperative or impaired; the maximum muscle tension may be reduced, and the gradation in force may be less refined. Where the primary defect in the MU is in the MN (a neuropathy), the

number of viable MUs will be reduced. This can be contrasted with the case where the primary defect is in the muscle fibers (a myopathy) and the number of fibers per MU is reduced rather than the number of MUs. The system possesses a high degree of plasticity, enabling healthy muscle fibers which have lost their MNs to become re-innervated by the remaining healthy MNs. The effect is that the number of MUs can decrease while the number of operative muscle fibers, and therefore the maximal muscle tension, remains constant.

Indications that a disorder strikes fibers in groups all belonging to the same MU is usually seen as evidence of a disorder of the nerve supplying the MU, rather than a myogenic disorder which would be expected to affect fibers randomly, by position in the muscle, or by some other criterion. It is very difficult, however, to identify all fibers innervated by the same MN, or to otherwise obtain evidence that a disorder has selectively affected entire MUs. A technique for estimating the number of functional MUs would provide a means for assessing the involvement of whole MUs in the aetiology of a disorder.

The concept of the MU has become important in the study and assessment of disorders of the neuromuscular system; in particular, the number of viable MUs has been used widely as evidence for distinguishing neuropathies from myopathies. A technique, such as the one presented in this thesis, of counting the number of functional MUs has tremendous importance in the field of neuromuscular physiology. As expressed by Goodgold (1983), "An accurate assessment of the number of active motor units within a muscle would be of inestimable value in the investigation and detection of neuromuscular disease". As a research tool, a counting

method provides a means of quantifying the effects of a disorder or treatment upon the MUs. In a clinical setting, it can be used for pre-operative screening to identify patients with clinically silent de-nervation (Delbeke, 1982), as a diagnostic tool, or as a procedure for plotting the progress of a disease.

1.1.2. Physiological Basis of Electromyography

The MU counting method to be described in this thesis is based upon electromyography, and a brief introduction of this topic is appropriate at this point.

Transmission of information along nerves is an electrical phenomenon, as is the primary event which initiates muscle contraction. Electromyography (EMG) is the measurement and study of the electrical signals associated with the activity of muscles and peripheral nerves. The surface membrane of a nerve or muscle cell normally has an electrical potential across it; this potential is generated by the distribution of ions across the semi-permeable cell membrane. These polarized cell membranes have the additional characteristic of being able to propagate a wave of depolarization along their surface. This wave of depolarization, the AP, appears as a moving dipole and will originate a potential which can be detected by electrodes positioned near the membrane. The characteristics of the detected signals can be analyzed to provide some information about the physiology, morphology, and control of the tissues which produced them. Thus EMG is a clinically useful technique for diagnosing disorders or for assessing the value of a course of treatment. EMG has

also been widely used as a control signal for prosthetic devices, and in bio-feedback studies.

1.1.3. Advantages of Evoked Versus Voluntary Potentials

The use of EMG generally falls into one of two catagories: evoked EMG responses, and voluntary EMG. When the information sought pertains to the central nervous motor control system, then voluntary EMG will incorporate the information required, and an evoked response might be used simply to provide a reference for calibration purposes. When the information desired involves the MUs themselves, then it is desirable that the influence of the central control system upon the data be minimized.

When attempting to characterize the dynamics of a two port system, it is common engineering practice to analyze the output response to a known input. By using direct stimulation of the muscle (or the nerve supplying the muscle) precise control of the muscle command signal can be asserted. This permits the use of stimulus or data acquisition schemes not possible under voluntary control, and limits the effects of factors such as subject compliance, central nervous system fatigue, and defects in the central motor control system.

Stimuli which are smaller than a specific amplitude - the motor threshold - will not produce any physiological response. As the stimulus amplitude is increased, a MN will be excited and a motor unit action potential (MUAP) signal will be generated as the muscle fibers in the MU are excited. If the stimulus amplitude is large enough, <u>all</u> of the MUs will be stimulated, and their MUAPs will be superimposed to produce the maximally evoked potential (MEP).

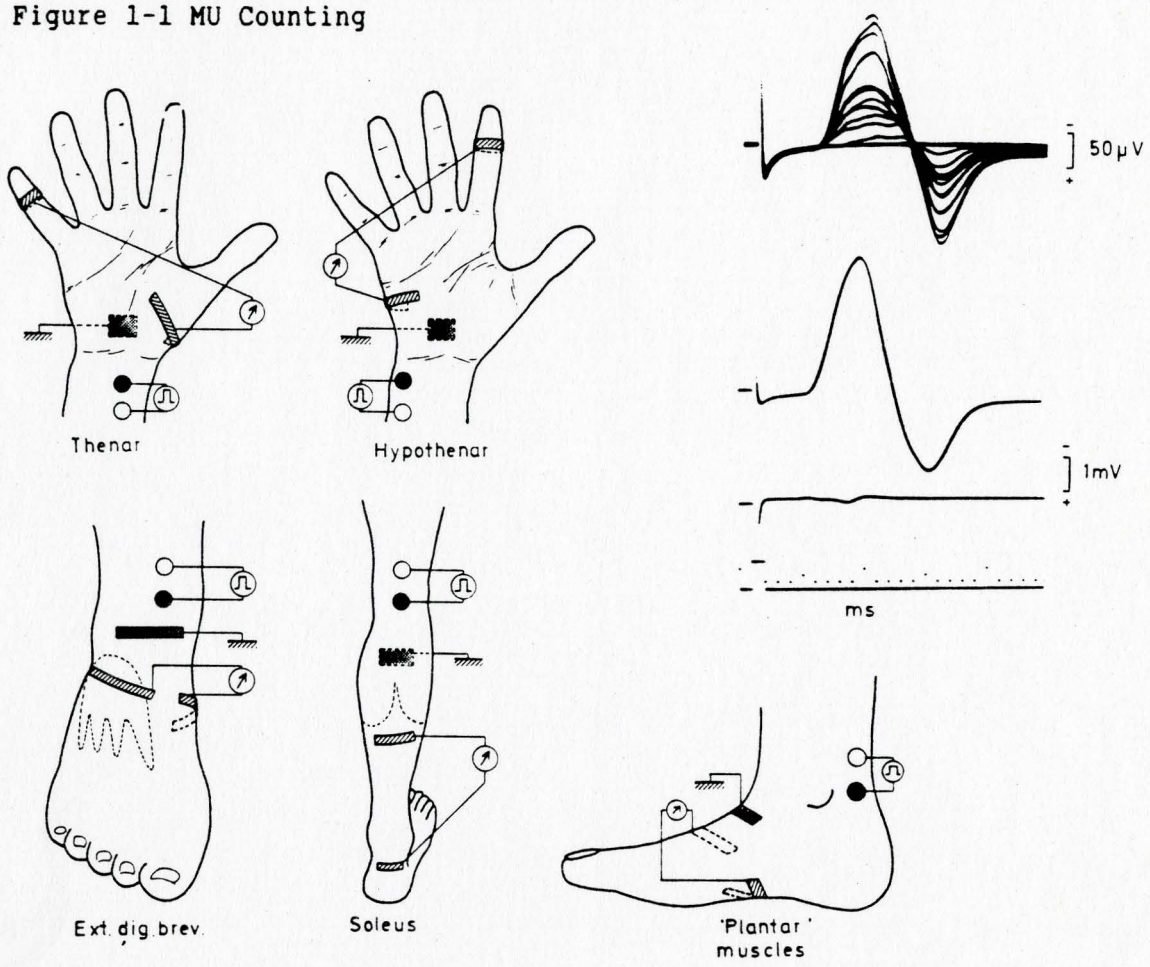
1.2. The Development of the McComas Method

1.2.1. A Summary of McComas's Method

In 1971, McComas and his colleagues introduced a relatively non-invasive, electromyographic technique for estimating the number of functional MUs in a human muscle. Before this time, a non-destructive method of assessing the loss of whole MUs was not available. The method is to estimate the peak-peak amplitude of the mean MUAP and of the MEP; then, the expression for the mean is rearranged such that the estimate of the number of MUs, N, is obtained by dividing the sum of all of the responses by the average response: N = MEP/(average MUAP).

The estimate of the average MUAP is obtained by using graded stimuli to obtain a composite response which is generated by the simultaneous firing of a small number of MUs. While the stimulus is increased to the level required to evoke this response, a discrete number of responses are identified; this is assumed to be the number of MUs that have been recruited. Dividing the amplitude of the composite response by the number of MUs recruited to generate it gives the mean MUAP amplitude. The MEP is found by increasing the stimulus amplitude until no further increase in the response occurs. Figure 1-1 presents an example of the incremental signals, the MEP, and the calculation of the MU count estimate.

Figure 1-1 MU Counting



The experimental arrangements for estimating numbers of MUs in human muscles (or muscle groups).

Estimation of the numbers of functioning motor units in the left extensor digitorum brevis muscle of a 25-year-old healthy female subject. At top are shown the eleven increments recorded as the stimulus was gradually increased from a sub-threshold value; each of the first eight increments had been superimposed several times. The trace below shows the largest potential that could be recorded from the muscle when stronger stimuli were applied; this is referred to as the maximum evoked potential (MEP). The next trace shows that the dorsal interosseous muscles made very little contribution to the recorded potentials. In this subject, the MEP was 7.1 mV and the mean motor unit potential amplitude was 34 μV; by division, the number of motor units was estimated to be about 208.

(Adapted from McComas, 1977)

The counting technique just described has become part of the standard set of EMG patient assessment tests conducted at a number of clinics. McComas and other researchers have used the above technique to study various neuromuscular disorders. The technique has been used to provide evidence for the sick motor unit hypothesis (McComas, 1971a), by providing a means of assessing the involvement of the nervous system in afflictions which are conventionally categorized as myopathies.

1.2.2. Summary of Criticism

The MU counting technique proposed by McComas has limitations and presupposes that certain assumptions are correct. Most of these limitations and assumptions were presented in the original paper describing the procedure, and there continues to be much discussion and criticism regarding the validity of the method. The details of these criticisms and likely means for their resolution are given in Chapter 2; but in general, the comments fall in one of five groups which are briefly outlined here.

Some of the discussions consider the possibility that the MUs recruited to estimate the average MUAP are non-representative of the MUs in the muscle. Another debate concerns the difficulty in obtaining error-free identification of the increments in the response corresponding to the recruitment of each new MU; concerns here are that the smaller discrete increments will be masked by noise, or that instability of the excitation thresholds will render invalid the assumption that a MU will continue to fire with any stimulus level greater than or equal to the level at which it was first recruited.

Another controversy involves the manner in which the MUAPs summate, and the suggestion that area is a preferable feature for use in the calculation of the estimate. Also at issue is the assumption that the point of stimulation is chosen to ensure that all MUs of the test muscle will be stimulated when the MEP is obtained, and that interference from other muscles is minimal. Finally, the questions of accuracy and precision: validating the method, estimating the confidence interval for a particular patient's MU count, and obtaining an expected normal range of values for various muscles - considerations which enable the clinical use of the method in patient assessments or diagnoses.

1.2.3. Previous Steps Towards the Automation of the Method

Although Shine (1982) described a micro-computer based MU counting system, no attempt has been made to automate the MU counting procedure; however, equipment has been used to assist the operator by providing additional enhanced graphical displays of the EMG signals.

A system developed by Ballantyne and Hansen (1974a) used a computer to automatically calculate and display various statistics, and to maintain and display a set of templates with which responses could be visually compared. The statistics included the area and peak to peak amplitude of the responses, and the area of the difference between the response and the closest template. As used in this thesis, the term "template" refers to the set of amplitude values obtained by sampling the ensemble average of a number of continuous-time signals. In a subsequent study (Ballantyne and Hansen, 1974b), the system was enhanced to display the MUAPs obtained using serial subtraction of the templates. The control

of the stimulus, and all decisions regarding the identification of increments, rejection of signals due to noise etc. were resolved by the operator. Their system served as a memory aid for the operator, relieving him of the exacting task of recalling the previous responses evoked.

Another application of technology to the method has been the use of ensemble averaging to detect small MUAPs. Panayiotopoulos et al. (1974) have used a microfilm reader to accomplish a visual form of signal averaging to extract small increments buried in noise. In this manner it was possible to identify small increments in the response caused by the addition of a small MU to the group of firing units and thus prevent the final count from being low as a result of missing the smaller units.

1.3. Objectives of This Thesis

1.3.1. The Role of an Automated MU Counting System

The manual implementation of the McComas MU counting method has inherent limitations which have impeded the development and acceptance of the method. One such limitation has been the observer-dependent nature of the test. The clinician is required to make various judgments as he manually performs the test procedure, these decisions apply often vague criteria to non-deterministic signals. This has made difficult the comparison of results of tests performed by different clinicians - even clinicians at the same facility. An automatic implementation of the method must provide results which are independent of the particular clinician conducting the examination.

Because of the computer's ability to acquire and process large amounts of information quickly, an automatic implementation can reduce the test duration or increase the amount of data acquired for use in producing the assessment. This will result in a cost/time savings, or an increase in the accuracy of the result which is, as mentioned above, based upon non-deterministic signals. An automatic implementation can permit further improvements in the accuracy or reliability of the results through increased accuracy of the measurements, increased signal processing, or modifications to the basic procedure which would be impractical in a manual implementation.

1.3.2. Thesis Objectives

The objectives of this thesis have been to use a general purpose computer to implement the MU counting method developed by McComas. The resulting system should be suitable for use in a research clinic environment, providing a flexible tool for clinical trials or for further development of the technique.

1.3.3. Summary of the Chapters

This chapter has briefly described the concept of the MU and the manual electromyographic technique proposed by McComas to estimate their numbers in a human muscle. In Chapter 2, the MU is considered in greater detail to permit a clearer understanding of the impact upon the method of variations in the characteristics of the MUs found in health and disease. Chapter 3 examines the technical aspects of the electrical phenomenon which occur during stimulation of nerves, and which modify the signal

measured at the surface of the skin. The principles of the automated counting method are described in Chapter 4 along with their underlying rationale. Chapter 5 includes a sample of the output produced during administration of the test procedure, and presents the results of preliminary trials using the automated system on a small number of normal subjects. Finally, Chapter 6 contains an assessment of the thesis in terms of the stated objectives, and proposals for further work. The details of the software implementation of the system are left to the appendix.

CHAPTER 2.

THE MOTOR UNIT: PHYSIOLOGY AND MEASUREMENT

In Chapter 1, the basic concepts of the motor unit, electromyography, and the McComas counting technique were presented. The material in this chapter describes the aspects of physiology and electrophysiology which are relevant to the techniques to be discussed. This is followed by a review of previous work in the field of motor unit counting methods, and the attendant problems which have been addressed in the literature. The purpose of this chapter is to provide the reader with the background required to comprehend the discussions that are to follow.

2.1. Anatomical Description

2.1.1. General Definitions

This thesis describes investigations of human striated (as opposed to smooth or cardiac) muscle and the neurons which innervate it. Figure 2-1 depicts the components of a MU. The only factor which is clearly common to all of the muscle fibers in a MU is the influence of the MN. The MN axon propagates APs, and also transports physical material to the muscle fibers from the cell body located in the spinal cord. This axoplasmic transport, which also moves chemicals in the reverse direc-

tion, is essential to the maintenance of the neuron, the muscle fibers, and the Schwann cells.

As each motor axon enters the target muscle, it divides into branches which terminate at the motor end-plate (Desmedt, 1981). The result is a one-to-one match of terminal branch and muscle fiber. At the myoneural junction, the transmission of an excitatory signal is achieved by the release of acetylcholine from the terminal branch (Katz, 1966); the acetylcholine diffuses across the narrow synaptic gap and initiates an AP in the muscle fiber. This AP quickly propagates bi-directionally towards the tendons, initiating the contraction process simultaneously over the entire length of the muscle fiber.

An AP travelling distally along a MN axon will normally result in the coordinated contraction of all of the fibers in that MU. Various disorders can modify the mechanisms in this complex sequence, thereby altering the characteristics of the parameters which are employed to estimate the number of MUs in a muscle. This is discussed more fully later in this chapter.

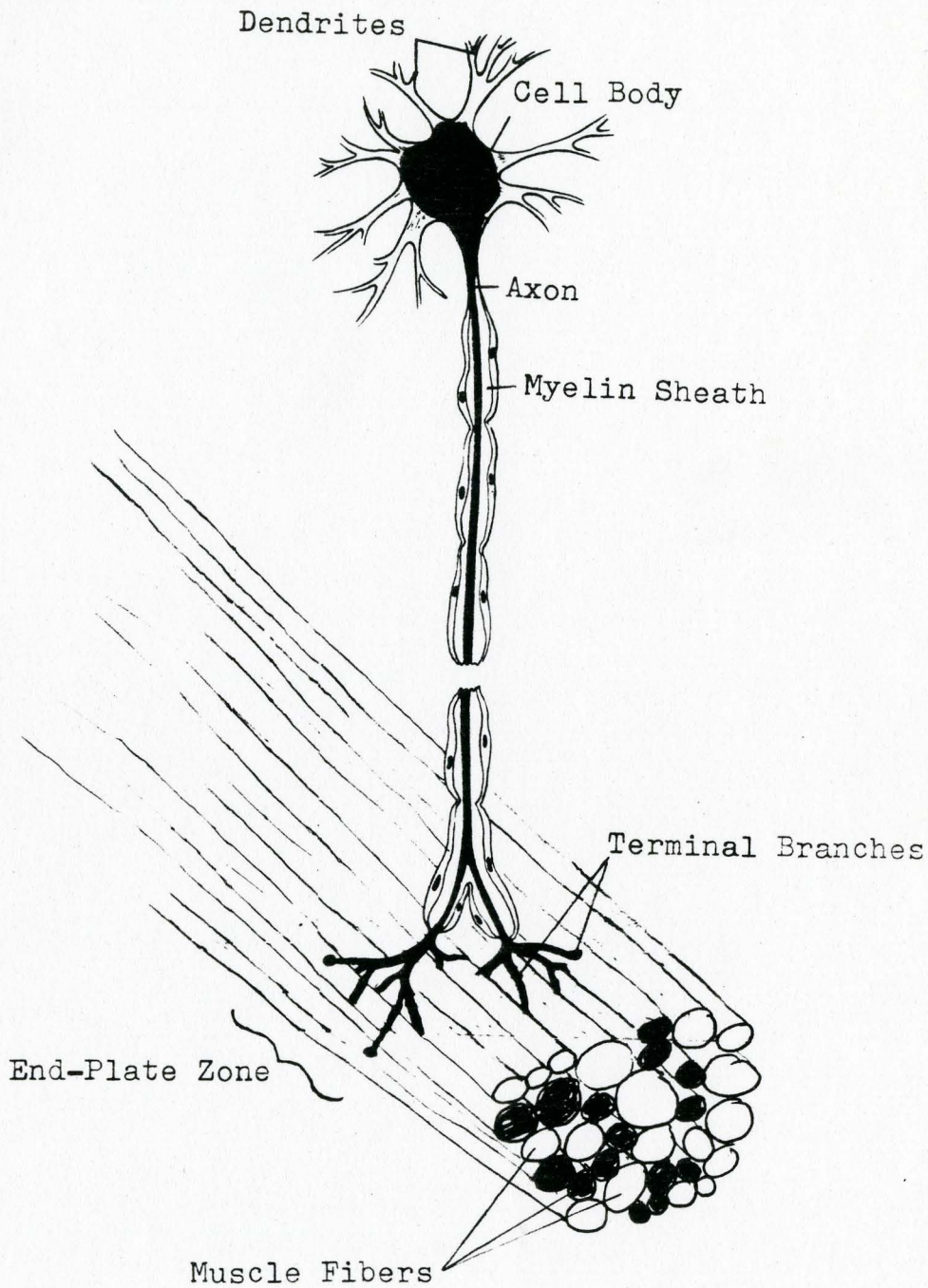
2.1.2. The Muscle Fibers

A muscle is composed of many fibers, each lying parallel to one another and generally extending the full length of the muscle. The muscle fibers of one MU are usually intermingled with fibers of other MUs as illustrated in Figure 2-1. Using physiological and/or biochemical properties, it is possible to classify muscle fibers into a number of distinguishable groups (Edgerton, V.R. and Cremer, S. in Desmedt 1981). However, all of the muscle fibers within a MU fall into the same

classification, a phenomenon known as homogeneity of the MU. It has been shown that MUs possess a good deal of plasticity with respect to the number of fibers they include, and the fibers themselves are able to alter their properties (Kugelberg, E. in Desmedt, J.E. 1981).

A muscle fiber which loses its MN becomes available for innervation by any other MN which is able to extend a terminal branch to it. If the fiber is successfully reinnervated, it will assume the characteristics of the other fibers innervated by that nerve and MU homogeneity will be maintained. Normally, MU muscle fibers are widely scattered within a large territory, and territories of different MUs overlap one another. Reinnervation results in an increased number of fibers in a MU without an appreciable increase in the extent of the MU territory. This result of collateral reinnervation - an increase in the fiber density of the MU - is often accepted as evidence that a disorder is neurogenic in origin.

Figure 2-1 The Motor Unit (MU)



2.1.3. Nerves and Axons

Nerves are made up of many axons extending from neurons whose cell bodies are located either in the ventral horn of the spinal cord (efferent, or motor neurons) or the dorsal root ganglion (afferent, or sensory neurons). Nerves are protected from physical damage over most of their length by following a route deep within tissues and close to bones; this also makes them difficult to access for stimulation. They have a tree structure in which fibers destined for different muscles and organs branch off at points along the length of the nerve. Final branching of a motor nerve occurs just as the nerve is entering a muscle, so that the individual axons find their way into regions of the muscle and provide a one-to-one synaptic relation of nerve endings to muscle fibers. Branching of nerves can cause difficulty in finding a stimulation point which results in the specific and complete access to the muscle to be tested.

The MNs which are to be enumerated are alpha motor neurons. Alpha MNs are responsible for innervating the extrafusal muscle fibers which produce the force apparent during a muscle contraction. The gamma MNs serve the intrafusal muscle fibers which are part of the muscle-length detecting organs, the muscle spindles, found within a muscle. As well, the nerve serving a muscle contains sensory fibers from the muscle spindles and the Golgi tendon organs (force detectors).

A cross-sectional view of a peripheral nerve reveals a wide range of axon diameters. It is expected that the smaller (<8μm diameter) myelinated fibers are gamma MNs (Guyton, 1982). The larger (>8μm dia-

meter) myelinated fibers are evenly divided between alpha MNs and sensory fibers (McComas, 1971a). The size of the fibers affects the speed of conduction and also the excitability of the fiber; smaller fibers have a slower conduction velocity and are not as easily depolarized by a given current pulse (discussed in Chapter 3). Myelination of the axon gives rise to saltatory conduction which is faster than continuous conduction in non-myelinated fibers of the same diameter.

When a nerve is artificially stimulated, sensory nerves will be excited as well as the motor nerves. The stimulation of a sensory fiber can indirectly excite the motor fibers via a reflex response - the H-reflex (Webster, 1978). Impulses travelling towards the spine along the gamma MN will excite the alpha MN with which they have synaptic connections in the spinal cord. This is an artificial manifestation of the mono-synaptic stretch reflex; a closed-loop system normally used to maintain a constant limb position under varying loads. The result of the reflex is that, in addition to the direct response of the muscle to stimulation, there may be a second response occurring after a short (approx. 50mS) delay.

2.1.4. The Neuro-muscular Junction

When an AP arrives at the MN axon terminal, packets of acetylcholine are released. These chemical transmitter packets diffuse across a narrow gap to the membrane of the muscle fiber, causing local end-plate potentials which are integrated over the membrane surface and evoke an AP. Normally, 2 to 5 times as much transmitter is released as is required to excite the post-synaptic membrane - a large safety factor which

ensures that the AP will successfully traverse the synaptic junction. During times when the MN is silent, the MN terminals release packets of acetylcholine at random intervals. This causes miniature (sub-threshold) end-plate potentials to appear on the post-synaptic membrane (Katz, 1966).

Most of the muscle fibers (approximately 98%), have only a single neuromuscular junction (Guyton, 1982). This junction usually is positioned equidistant from the ends of the fiber. The set of neuromuscular junctions occurring in the muscle constitute the end-plate zone. The end-plate may be either spread over the length of the muscle or contained within a well defined region; in muscles where it is restricted to a narrow region, the APs evoked from all of the fibers will be synchronized and arrive simultaneously at the recording electrodes.

2.2. Electrophysiological Description

2.2.1. The Concept of Excitable Membranes

Both muscle and nerve cells are enclosed in excitable membranes - excitable because when the membrane is depolarized beyond a threshold a positive feed-back mechanism will cause a transient reaction to occur. This reaction, the AP, serves to transmit sensory and command data within an organism and plays a central role in the initiation of muscle contraction. Relevant to the MU counting technique discussed here are the measurable potentials resulting from changes in the membranes of muscle fiber cells, and the effects on the excitable nerve fiber axon membranes of an externally applied stimulus potential.

2.2.2. The Ionic Basis of the Resting Potential

For an excitable cell in the normal, resting state, there is a potential difference maintained across the cell membrane, with the internal medium approximately 50 to 100 mV more negative than the surrounding environment. This trans-membrane potential is caused by an imbalance in the concentrations of ions across a semipermeable membrane. The concentrations of the ions most important to the determination of this potential are given in Table 2-1.

Table 2-1: Ionic Content of Mammalian Skeletal Muscle

Ionic species:	Na^+	K^+	Cl^-	
Intracellular	10	160	3	(mEq/l)
Extracellular	145	4	114	(mEq/l)

(From McComas, 1977)

These concentrations are maintained by a sodium-potassium "pump" which transports the ions across the membrane and against the diffusion gradient at the expense of metabolic energy.

It is the relative ionic permeability of the membrane which creates the membrane potential. In its normal resting state, the cell membrane restricts the passage of sodium while permitting the other ions to move relatively unimpeded. The potassium and chloride ions tend to diffuse down their concentration gradients, creating a charge imbalance due to an excess of sodium ions trapped outside the cell. In the steady state condition, an equilibrium is reached where the ionic movement due

to the diffusion gradient is exactly countered by the electromotive force resulting from the charge imbalance. The resulting ionic distribution is called the Donnan equilibrium, and the potential difference due to the unequal distribution is given by the Nernst equation.

The Nernst equation (at 37°C):

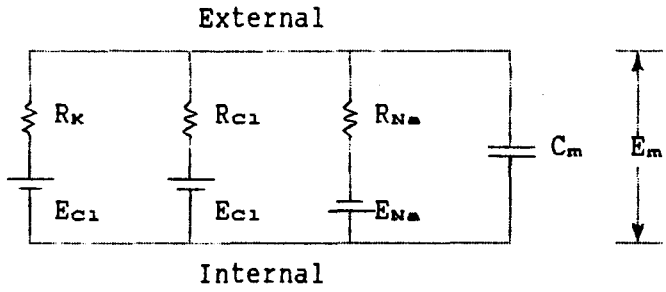
$$E_A = \frac{60}{n} \log_{10} \frac{[A]_o}{[A]_i} \text{ mV}$$

Where: n is the valence of ion "A"

$[A]_o$ and $[A]_i$ are the extra- and intracellular concentrations of ion "A"

Calculating the potential using the ionic concentrations from Table 2-1, the Nernst equation gives an equilibrium potential of -95mV for chloride or potassium and +70mV for sodium. The actual membrane potential will lie between these two values according to the relative permeability of the membrane to the ions. An electrical circuit analogue of the membrane illustrates this dependence of the trans-membrane potential on the relative ionic conductances (Figure 2-2). The circuit consists of three channels, one for each of the ions. The batteries represent the equilibrium potentials for each ion; the resistors represent the membrane leakage resistance. The potential difference across the capacitor C_m, representing the potential across the membrane, will be closest to the voltage generated by the channel with the lowest resistance.

Figure 2-2 : Electrical Circuit Analogue of the Membrane



(From Katz, 1966).

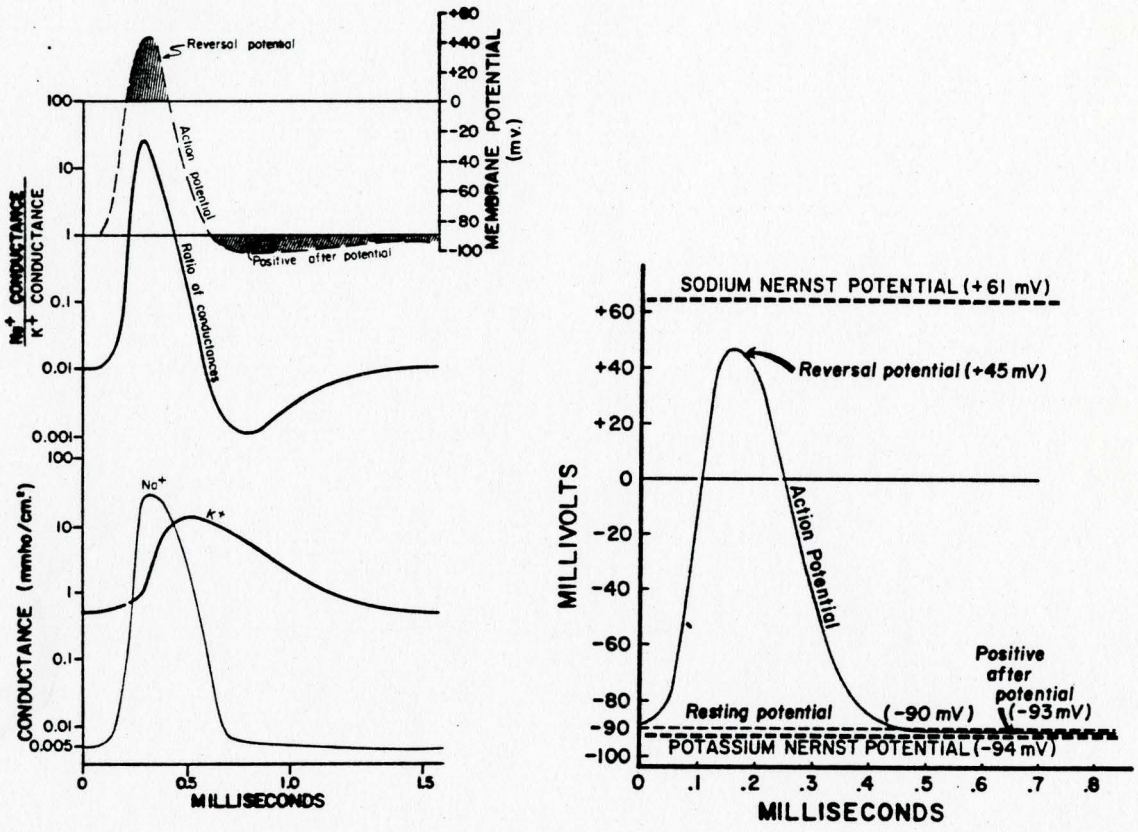
As discussed above, while in the resting state, the membrane is relatively permeable to potassium and chloride; thus the resting potential is closer to the equilibrium potentials for these ions. Any event or condition which alters the relative ionic conductances will alter the membrane potential; the AP results from the fact that the ionic conductances are themselves a function of the membrane potential and of time.

The AP is an all-or-nothing event resulting from a regenerative process which causes each discrete area of membrane to behave much like a monostable binary circuit: a triggering signal will cause a transition from a stable state to a quasi-stable state. After a fixed time, the membrane (circuit) returns to its stable state. While some characteristic of the triggering signal must exceed a threshold level to initiate the transition, both the qualities and duration of the quasi-stable state are independent of the triggering signal characteristics.

When an excitable cell membrane is depolarized by some external influence, the membrane permeability to sodium will increase. This increase in the sodium permeability will move the equilibrium potential in the positive (depolarized) direction. If the membrane is depolarized

beyond a certain level (the threshold) this positive feedback system will become unstable and the depolarization will continue independently of the external influence. Thus, during the AP the ionic conductance of sodium undergoes a transient increase, thereby temporarily drawing the membrane potential closer to its equilibrium potential of +70mV. At the same time as the sodium conductance is returning to its normal (low) state, the conductance of potassium increases and provides a further restoring influence on the membrane potential. This temporary increase in the potassium conductance causes a hyper-polarization of the membrane and makes initiation of another AP more difficult; this duration of a higher threshold is called the refractory period. The sequence of events occurring during a typical AP are shown in Figure 2-3.

Figure 2-3 The Action Potential



Changes in sodium and potassium conductances during the course of the action potential. Note that sodium conductance increases several thousand fold during the early stages of the action potential, while potassium conductance increases only about 30 fold during the latter stages of the action potential and for a short period thereafter.

Also shown is the relationship of the action potential to the potassium and sodium Nernst potentials.

(Adapted from Guyton, 1982)

2.2.3. Propagation of the Action Potential

The propagation of the AP along a nerve or muscle fiber is a result of an AP occurring at one section of membrane acting as the triggering influence for neighboring areas of membrane. An AP initiated in any region of a cell's membrane will result in local currents induced by the difference in potential between adjacent sections of membrane. These local currents depolarize the neighboring areas of membrane beyond the threshold required to initiate an AP in a resting membrane, but not sufficiently to initiate an AP in a membrane which is in the refractory period. (Katz, 1966). In this manner, the AP will propagate along an excitable cell membrane, with the refractory period normally ensuring unidirectional, echo-free transmission. Externally applied stimulation normally results in a bi-directional transmission because none of the membrane surrounding the stimulation site is in the refractory period.

The speed of propagation is a function of the cable properties of the fiber; fibers with a lower attenuation will propagate the AP faster. This is because the depolarizing influence of the local currents extend a greater distance along the fiber. The role of myelination is to improve the cable properties of the axon. The speed of conduction varies from 130 m/S for large myelinated axons down to 0.5 m/S for small, unmyelinated axons. APs propagate much slower in muscle fibers: about 3 - 5 m/S (Guyton, 1982). The duration of the AP (typically 1 - 5 mS) and the maximum discharge rate (less than 1000 impulses/s) are determined by the

duration of the transient increases in membrane conductance of sodium and potassium.

The MU counting technique is an application of the electrophysiology just described in two respects: the moving wave of depolarized membrane generates the EMG signal which is exploited in the MU counting technique, and secondly, external (electrical) stimulation of nerve fibers is used to obtain a controlled, reproducible response from the muscle fibers. Both of these applications are covered in more detail in Chapter 3. The next section describes the effects of pathologies on the neuromuscular system.

2.3. Disorders of the motor unit

2.3.1. The Distinction Between Neuropathic and Myopathic Disorders

There is considerable evidence which shows a close relationship between the states of health of nerve and muscle fibers, therefore the cause and effect of a disorder is often difficult to assign conclusively. Diseases which involve the two types of fibers are customarily classified as a neurogenic, myogenic, or a neuromuscular junction disorder, according to the site of greatest abnormality (McComas, 1977). A decrease in the number of MUs, and/or the presence of very large MUAPs (resulting from collateral reinnervation) is usually taken as evidence of a neuropathy. Evidence of a myopathy would be a decrease in the average size of the MUAP, although it is possible for a neuropathy to present these symptoms if the MNs are either unable to maintain some of their terminal

branches or to sprout new branches as required during the process of collateral reinnervation (McComas, 1971x). Conversely, a myopathic disorder which is selective with regard to fiber type may result in a neuropathic pattern of muscle involvement and loss of MUs (Eisen et.al., 1974).

2.3.2. The Effects of Disorders on the EMG signal

Having discussed the characteristics of healthy MUs, various pathologies and their effects on these characteristics are now discussed. Note that a disorder is not considered if its impact is restricted to the cellular processes involved in transducing the electrochemical events into mechanical force. For example, in the case of fatigue of the muscle fibers, the electrical events which initiate muscle contraction normally can continue long after the metabolic processes are able to support contraction, and the muscle fibers cease to contract. Thus the EMG signal, and the MU counting method described here, will not reflect deficiencies in a muscle's ability to contract when properly mobilized.

[1]Acute partial denervation will decrease the MEP and the number of functional MUs, but will not alter the mean MUAP. Partial denervation followed by collateral reinnervation by the remaining healthy MNs results in a clustering effect which produces a larger mean MUAP. If the demand for reinnervation is not too extensive, the MEP will recover to the pre-denervation amplitude. During the reinnervation, increased jitter in the latency of the individual fiber MUAP may occur because of a decrease in

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[1] Except where noted, the information in the remainder of this subsection is primarily from McComas, 1977.

the safety factor at the newly formed neuro-muscular junctions (Desmedt, 1981); this excessive jitter may cause stimulus to stimulus variations in the shape of the MUAP recorded at the surface. Ballantyne and Hansen (1974a) have suggested that partial denervation and reinnervation may be "part of the normal dynamic process of wear, tear, and repair that occurs in muscle". Thus, it may be that the number of MUs within a particular muscle can vary with time, and that some evidence of denervation in a muscle at a particular time must be considered normal.

A disease of myopathic origin should affect the muscle fibers at random within a muscle, and therefore result in MUs having a reduced MUAP. The MEP will also be reduced, while the number of MUs remains constant until the disease is greatly advanced. A decrease in the mean fiber diameter, such as by disuse atrophy, will have similar effects upon the EMG signal. A disorder such as Duchenne muscular dystrophy will tend to produce poly-phasic MUAPs. Myotonia is a condition where defects in the muscle fiber membrane create a situation of hyper-excitability and results in continuous firing after an initial excitation. This would make controlled external stimulation difficult.

Eaton-Lambert syndrome causes a decrease in the safety factor at the neuromuscular junction resulting in a transmission failure occurring at a proportion of the junctions, that is, a "fragmentation of motor units" (McComas, 1977). Thus the MUAP will be inconsistent in shape or amplitude from one stimulation to the next in a manner similar to that which occurs during collateral reinnervation discussed above. Myasthenia gravis, another disorder of the neuro-muscular junction, results in a

decrease in the evoked EMG response with repeated stimuli or following a maximal voluntary contraction.

The background EMG noise level may be increased by a number of disorders. The subject may be non-compliant, or unable to voluntarily relax the muscles in the region of the electrode. Fasciculations, spontaneous discharges of entire MUs, can occur in partially denervated muscles and in patients with thyrotoxicosis; fasciculations produce large irregular potentials. Fibrillation potentials are much smaller impulses caused by the spontaneous excitation of individual muscle fibers which normally occurs approximately one week after denervation. Linked potentials (Desmedt, 1981) are responses consistently occurring a fixed time after the firing of a MU, but not explainable in terms of reflexes. Their origin seems to be linked with the process of collateral reinnervation. The effect on the surface EMG is an increase in the "noise" signal for the period from 15 to 25mS after the MUAP.

Demyelination (as in Guillain-Barré syndrome) results in a decrease in the number of excitable MUs as the point of stimulation is moved proximally, and also an increase in the latency of those fibers which remain excitable. This may impair the reproducibility of the MU count estimate due to increased sensitivity to the positioning of the stimulating electrodes. In cases of nerve trauma resulting in a loss of function but no loss in the continuity of the nerve (neurapraxia), stimulation distal to the injury site will result in a larger MEP compared with proximal stimulation. This would also impair the reproducibility of the MU counting method.

It is clear that the effects of neuromuscular disorders on the EMG signal are complex. Some of these effects form the basis of a reliable MU counting system, while others reduce the reliability of a technique based upon the EMG signals.

2.4. Measurement of the Number of MUs

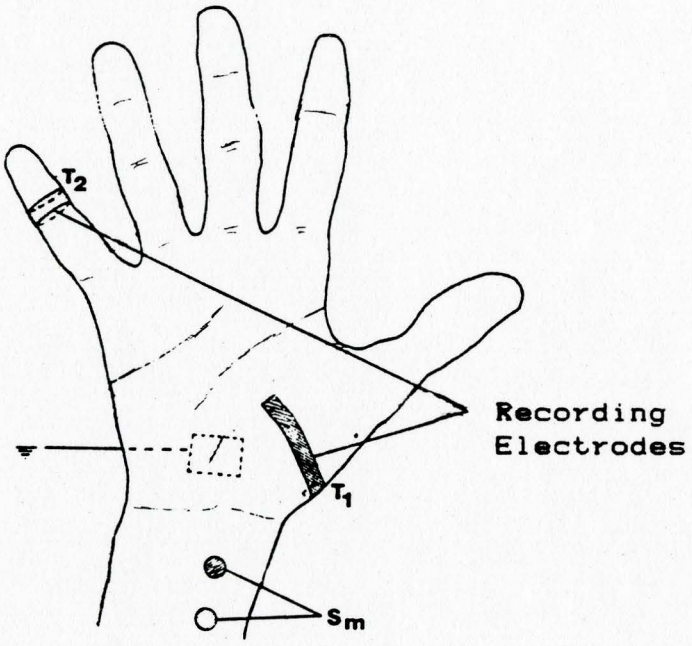
2.4.1. Application of the McComas Technique

The general method of estimating the number of MUs has been described in Chapter 1. This method amounts to linearly extrapolating the manner in which the first few MUAPs summate to estimate the number of MUAPs in the MEP. A variety of muscles have been studied by several researchers using this technique. The upper limb muscles studied include the thenar (bulky pad on the palm at the base of the thumb), the hypothenar (fleshy mass at the edge of the palm between the wrist and the base of the little finger), first dorsal interosseous (located deep in the palm of the hand), and the abductor pollicis longus (back of the wrist or lower forearm, becoming visible with the wrist completely extended against a force). The lower leg muscles previously studied include the soleus (calf muscle concealed by the gastrocnemius), the extensor digitorum brevis (high on the top of the foot, extends the toes), and the plantar muscles (three small muscles, the plantar interosseous, located in the arch of the foot). The frontalis muscle has also been tested.

The muscles selected for application of this estimation technique ideally share a number of features: The nerve which controls them must be easily accessed (close to the surface) at a point proximal to where the nerve begins to branch as it enters the muscle. The nerve must not contain axons destined for another muscle which, when stimulated, would evoke an EMG response which would be picked up by the surface electrodes. The muscle should be superficial and flat in cross-section to minimize signal degradation due to the intervening tissues, interference from intervening muscles, and variance in the MUAP amplitudes due to the variance in the depths of the MUs. The muscle should have a single, compact end-plate, this minimizes the variations in latencies to help ensure that the MUAPs will summate in a linear fashion. To provide a clinically more useful estimate, the muscle selected should normally have a small variance in the number of MUs in healthy subjects, and also be sensitive to the disorders of concern.

Figure 2-4 depicts the experimental arrangement used for the thenar muscle. The thenar eminence is formed by three muscles, the abductor pollicis brevis, the opponens pollicis, and the major part of the flexor pollicis brevis. A branch of the median nerve serves these muscles. The stimulating electrodes, two 10mm diameter discs 2 cm apart, are fastened over the median nerve. The stimulating voltage pulses are of adjustable amplitude with a pulse width of 50μS.

Figure 2-4 The Electrode Placement for the Thenar



Arrangement of electrodes as used by McComas and his colleagues for estimating numbers of thenar units. S_M, median nerve stimulating electrodes (cathodal distal); T_1 and T_2 are stigmatic and reference electrodes.

(Adapted from Sica, 1974)

Three silver strip recording electrodes are positioned as shown in Figure 2-4. The earth electrode is placed on the dorsum of the hand, when space permits, the earth electrode is typically positioned mid-way between the stimulating and recording electrodes. The positive-input electrode is positioned to completely cover the end-plate region, the reference (negative-input) electrode is positioned distal to the muscle body. The signals from the electrodes are amplified and band-pass filtered (2Hz to 1kHz), and displayed on a storage oscilloscope.

2.4.2. Restrictions and Criticisms of the McComas Method

A measurement system often determines a desired quantity (in this case, the number of MUs) by direct measurement of a more accessible measurand (the EMG signal) which bears a known relation to the desired quantity. The accuracy and precision of the measurement will depend on the correctness of the various assumptions modelling this relationship. The effects of various MU disorders upon the EMG signal have been described; there are clearly many alterations to the EMG signal which can occur to confound any relationship which might be used to infer the number of MUs from features of the EMG signal. Furthermore, the McComas MU estimation method is based upon an extrapolation of the relation between the number of MUs excited and a measured feature of the EMG response. Meaningful results will be obtained only if the model used in the extrapolation is appropriate for the range of values considered. Some of the assumptions fundamental to the McComas MU counting method are now described.

Fundamental to the technique is the estimation of the average MUAP response; it is assumed that the MUs sampled to obtain this estimate are representative of the population of MUs which contribute to the MEP. Various research groups have presented evidence which suggests that this assumption may be violated. Using isometric voluntary contraction, Brown & Milner-Brown (1976a) have detected what they believe to be larger MUs than are identified using graded electrical stimulation. They were unable to appraise the importance of this possible source of bias on the MU count estimate. Furthermore, the large responses which they observed may have actually been the summation of a number of MUs firing synchronously. Similarly, Feasby & Brown (1974) report late responses which they propose are MUAPs from single large MUs not represented in the sample of MUAPs typically acquired using graded stimulation.

Kadrie and his colleagues (1976) have found a direct correlation between the order of recruitment and the size of the MN; that is, they found that small MUs have lower thresholds to external electrical stimulation. The automatic system developed addressed this issue by providing an indication of the existence of a direct relation between the order of MU recruitment and the size of the MU.

Scarpalezos and Panayiotopoulos (1973a) have criticized the McComas technique on the grounds that small MUAPs whose amplitudes are near the noise level will not be identified and incorporated in the estimation of the mean MUAP response. They propose ensemble averaging as a method of minimizing this problem. Ensemble averaging will improve the signal to noise ratio by a factor of $N^{1/2}$, where "N" is the number of signals averaged, and the noise is uncorrelated with the sampling epoch.

The automatic system has incorporated ensemble averaging, and also an indication of the existence of an unidentified increment in the responses.

An assumption basic to the counting method is that each increment in the response to an increasing stimulus is a result of a single additional MU adding its MUAP to the responses of the other MUs which had been previously excited. However, it is possible that two or more MUs will have excitation thresholds which are similar such that they will always fire together and appear as a single large MU (Scarpalezos and Panayiotopoulos, 1973a), or fire in various combinations to produce a set of responses - a phenomenon called alternation by McComas (1971a). Alternation occurs because the excitation threshold of any particular MN is not an exact quantity, but is better described (Milner-Brown & Brown, 1976) as a range of stimuli values over which the probability of the MN firing increases from 0 to 100% - it is therefore possible for different MUs to have overlapping excitation thresholds.

Milner-Brown & Brown have found alternation to occur even at low stimulation levels (during the recruitment of the first MUs). They suggest that it may be possible to compensate for alternation mathematically; their proposed approach requires that a statistically large number of responses be sampled at each increment in the response. Using this scheme, they expect to evoke all the possible combinations of MUs whose probability of excitation was greater than 0% but less than 100% at each stimulus increment. The number of discrete increments in the response will be 2^n-1, with "n" being the number of alternating MUs. This method assumes that the discrete increments in the responses are actually due to

alternation and not to a variation in the effective stimulation level caused by movement of the stimulating electrodes. The automatic MU estimate system evokes many samples at each of the discrete increments, thus it is expected that any condition which would increase the alternation phenomenon would tend to cause the count to be overestimated. Alternation will be discussed again later in this thesis.

Scarpalezos & Panayiotopoulos (1973) have suggested that non-linear summation of the MUAPs due to variations in the latencies of the individual MUAPs may result in the smaller MUs being overlooked because they do not produce a change in the amplitude of the summated response. Also, nonlinear (non-algebraic) summation of the peak-peak responses results in a MEP with a reduced amplitude, and therefore result in an incorrect (low) MU count. Parry and his colleagues (1977) have offered another reason to expect a non-linear summation; they theorize that increased numbers of muscle fibers undergoing an action potential will increase the effective attenuation of the muscle tissue and decrease the amplitude of the surface EMG signal.

It would be beneficial to obtain an indication of the magnitude of the effects of the non-linear summation, and either consider it in the calculation of the estimate confidence interval, or correct for the non-linear summation. Such an indication can be made available by decomposing the responses evoked using graded stimuli to obtain the individual MUAP signals. These signals can then provide information regarding the variation in latencies and phases of the MUAPs, or an alternate estimate of the average MUAP.

Whereas McComas uses peak-peak amplitude as the signal feature used to calculate the MU count estimate, Ballantyne & Hansen (1974a) suggest that the area under the response curve is preferable. They present a theoretical demonstration indicating that area should provide an estimate which is less affected by variations in the MUAP latencies. The estimates based on base-peak amplitude and on area were both generated by the automatic MU counting system, thus permitting a comparison of the two features.

An accurate MU count requires that all MUs be stimulated to evoke the MEP, and that the electrical stimulation preserves the MU as the smallest group of muscle fibers which can be excited. Brown (1972) observes that anomalous innervation of a muscle combined with single point stimulation can inadvertantly result in the incomplete excitation of a muscle; his example is the flexor pollicis brevis (one of the three muscles of the thenar eminence) which may be supplied by the median, by the ulnar, or by both of these nerves. Kadrie and his colleagues (1976) have found that MU counts repeated using a more distal stimulation point can give a increased MU count and reveal smaller MUs. Their explanation is that axon branching can occur more than 100 mm proximal to the end-plate region of the small muscles of the hand. Thus, stimulation distal to this branching will isolate axon subunits rather than MUs.

The muscle to be tested must be carefully selected to ensure that it is representative of the general musculature under investigation. Denervation may occur in particular muscles in normal subjects (Jennekens et al., 1972), and these muscles will not be representative of the denervation of the subject's musculature when tested for other disorders.

It is necessary that the range of MU counts for control subjects be narrow enough to be useful for the intended purposes, and that the muscle fiber types and location (fast/slow, peripheral/proximal) be affected by the disorder considered. A partial solution may be to test more than one muscle.

2.4.3. Validation of the McComas Method

It would be desirable to validate the McComas technique by applying the test to a muscle with a known number of MUs; however, accurately enumerating the viable MUs in a muscle is difficult even using invasive or post-mortem methods. Some of the alternate means of assessing the number of MU are now discussed along with the extent to which their estimates agree with the estimates obtained using the McComas method.

The lack of "fullness" of the interference pattern obtained during maximal voluntary contraction is sometimes wielded as evidence of a reduction in the number of functioning MUs. Although non-invasive, this method is unsatisfactory on several accounts: it requires a motivated, compliant subject, it is highly subjective, and it is non-quantitative. McComas (1977) reports that a muscle which is determined to be 70% denervated using his method can escape detection with this method. Efforts to quantify the interference pattern tests (Rose & Willison, 1967) have not been completely successful because of the difficulty of identifying a feature of the interference pattern which is a function of the MU count and relatively independent of other confounding influences.

More invasive, but quantitative methods include microscopic examination of the peripheral nerves in conjunction with various methods

of identifying the number of alpha-MNs. McComas (1971a) obtained an estimate of the number of MNs from two cadavers by performing a histological examination of the nerve serving the extensor digitorum brevis and assuming that half of the large fibers were MNs. The resulting estimates, 365 & 280 MUs, fell within the range of 120 - 414 MUs obtained using his electrophysiological method on 151 normal subjects. Peyronnard (1975) undertook a bilateral comparison of the extensor digitorum brevis in the monkey; the left side was assessed using the McComas method, while the right side was assessed by excising the dorsal root ganglia and assuming that the remaining large fibers were alpha-MNs. His results show the McComas method to be consistently 10% lower than the histological estimate. Also using animal models, Eisen et al., (1974) used a modified electrode arrangement to enumerate the MUs in normal muscles, in muscles in which a myopathy had been induced, and in muscles which were partially denervated. Anatomical estimations agreed with the electrophysiological estimations, and as expected, only the denervated muscles showed a significantly reduced MU count.

Although there are many criticisms and weaknesses of the McComas MU counting method, there is no practical alternative currently available. The technique has "added a new dimension to the investigation and detection of neuromuscular disease" (Ballantyne & Hansen, 1974a). The following chapter examines the technical aspects of electrically stimulating nerves and measuring the EMG signal.

CHAPTER 3.

DEVELOPMENT OF AN AUTOMATIC MOTOR UNIT COUNTING METHOD

The preceding chapters have established the essential material required to appreciate the physiology and the electrophysiology of the MU. The McComas technique has been introduced along with a discussion of its weaknesses and practical limitations. The goal of this project was to develop an automated system which overcomes, or at least assesses the impact of, the technique's shortcomings.

This chapter describes the development of the system designed during this project. Sufficient detail is presented to allow an engineer to implement the system, or to evaluate and augment the methods used. The underlying rationale is explained in this chapter, whereas the description of the actual implementation can be found in the appendix.

This chapter begins by describing the equipment used to estimate the number of MUs, and then compares the automatic system protocol with the manual one used by other researchers. The resolution of the shortcomings inherent in the basic algorithm and the practical constraints encountered during the design of the automatic system are discussed.

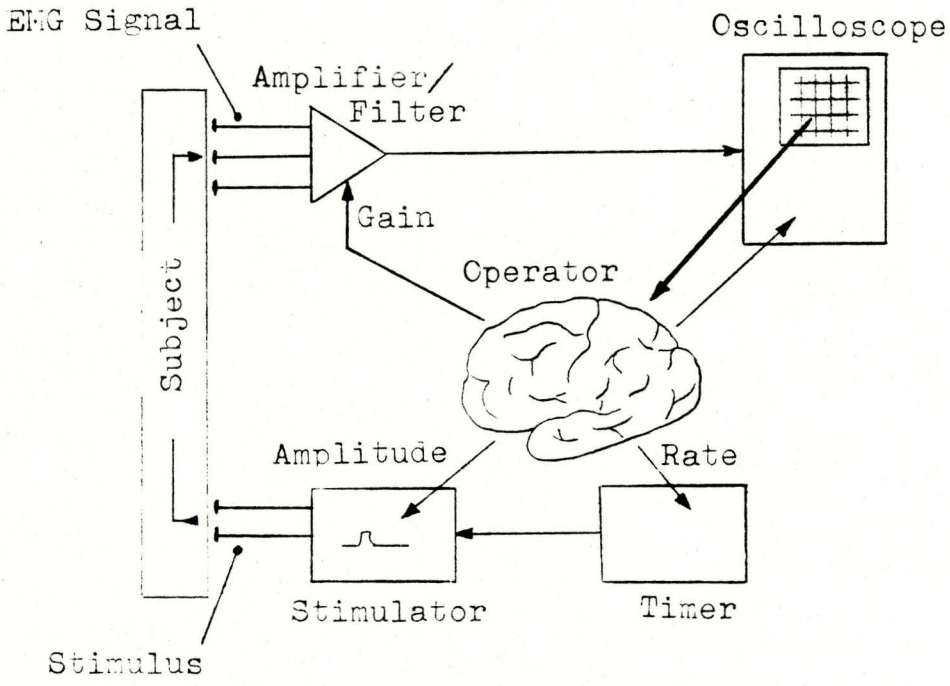
3.1. Description of the Equipment Used

3.1.1. Equipment Employed in Manual and Earlier Automatic Systems

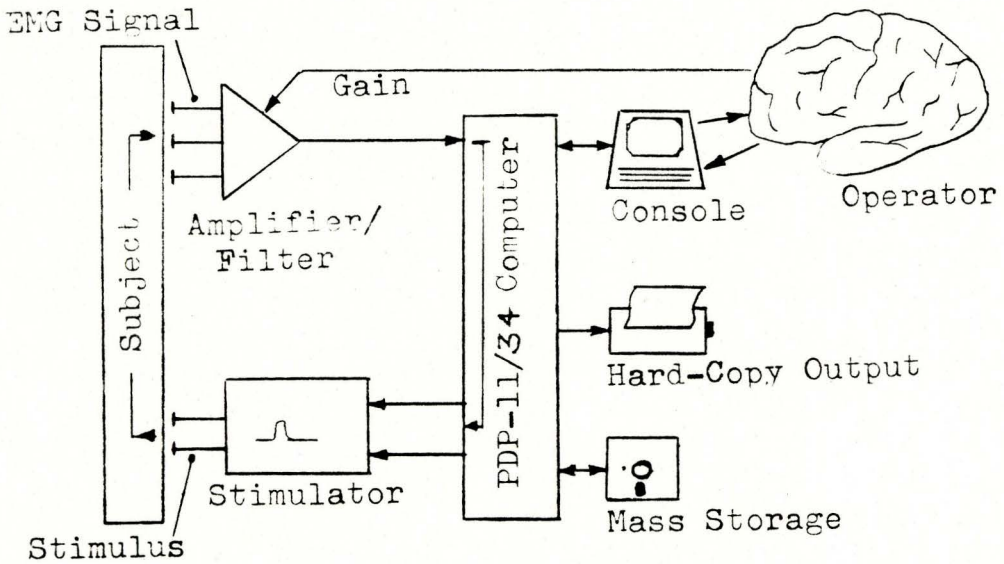
The equipment normally required to manually perform the test consists of a high voltage stimulator, a differential input preamplifier, a variable gain amplifier, a band-pass filter, an audio monitor, and a storage oscilloscope (see Figure 3-1a). An FM tape recorder is used to store the EMG signals if they are required for subsequent off-line processing. The oscilloscope screen is photographed if a permanent display of the signal is required.

Automation of the test implies that the computer must execute many of the tasks previously performed by the operator; thus the stimulator output voltage must be computer controllable, and the EMG response must be digitized. The audio monitor and the oscilloscope are not required with the automated system, but may be retained to enable the operator to monitor the progress of the test. A record of the sampled EMG signals can be kept on a digital mass storage device such as a diskette, thus eliminating the need for an analog recorder.

Figure 3-1 Motor Unit Counting Equipment



a) Equipment Required for Manual Estimation



b) Equipment Comprising the Automatic System

Equipment used in previous work to automate the McComas technique has been described by Ballantyne and Hansen (1974a). They interfaced a PDP-12 computer to an electromyograph which provided the stimulation and signal conditioning functions. A Digitimer clock generator was used to trigger the stimulator and the start of the signal acquisition. The stimulus amplitude was manually controlled, thus limiting the function of the computer to that of a signal analyzer. Kadrie and his colleagues (1976) implemented a similar system using a Hewlett-Packard 2100 computer. No record was found in the literature of a computer based system equipped to control the timing and stimulation aspects of the method in addition to performing signal acquisition and analysis functions.

3.1.2. The General Purpose Computer

The equipment used to implement this project is illustrated in Figure 3-1b. The core of the system consisted of a PDP-11/34 mini-computer equipped with a cache memory unit, a floating point processor, 128K words of RAM, dual 2.4M byte hard disk drives, dual 256K byte floppy disk drives, a Tektronix 4006 graphics terminal with a hard copy unit, a printer, and an LPS-11 laboratory interface unit. The PDP-11/34 central processor had a register-to-register ADD time of approximately 2.1 μS, and was fast enough to accomplish the required processing between periods of signal acquisition. Sufficient memory was available to accommodate 17 evoked potential templates, which was adequate for all of the tests performed.

The faster hard disk drives were employed to access the programs and data during the tests. After completion of the tests, the data were transferred to the floppy disk for archiving. The digital output port of the LPS-11 drove two 12 bit digital to analog (D-A) converters which controlled the stimulator; a single analog input channel was used to sample the EMG signal. A binary (Schmitt trigger) input was used to sense the closure of a push button switch. This input provided a command input which could be polled by software.

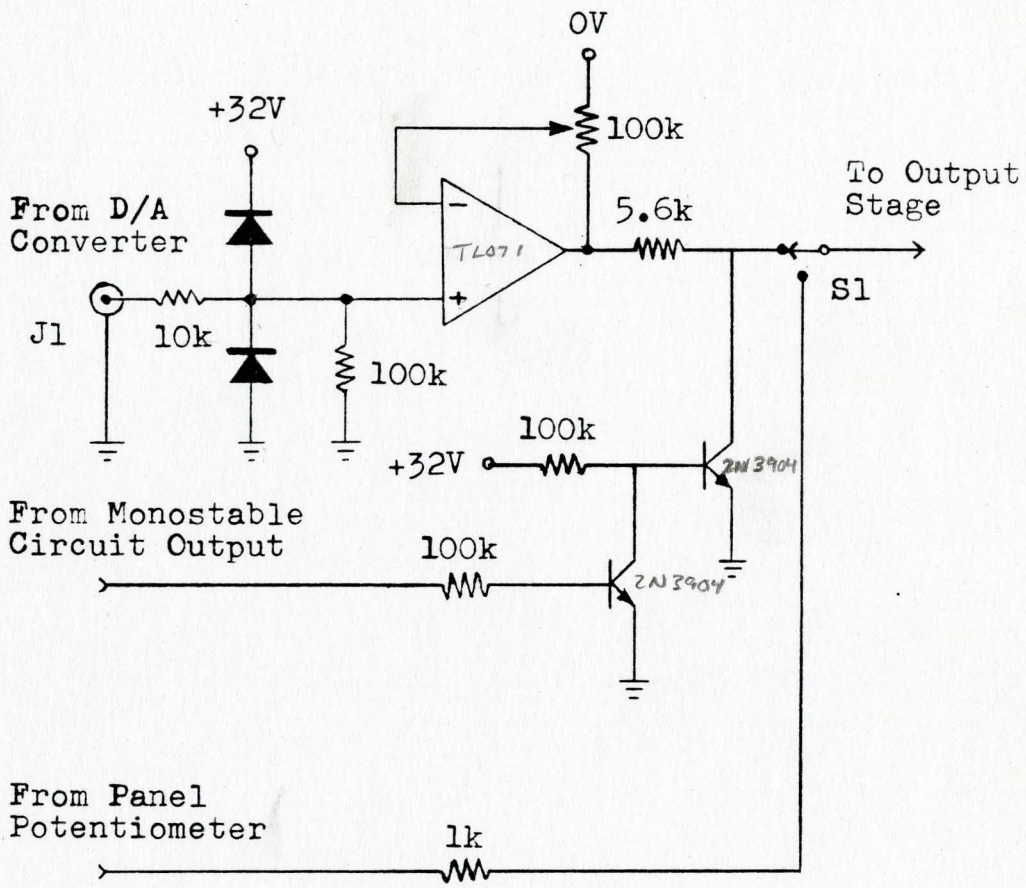
3.1.3. The Stimulator

The stimulator used during this project, the DEVICES 3070 stimulator, was designed to be triggered by a DEVICES DIGITIMER clock generator. To achieve computer control of the stimulator, one of the D-A converters' outputs was used to provide the trigger pulse which initiated the stimulus. By having the computer trigger the stimulator, not only could the stimulus rate be software selectable, but the pre-stimulus signal could be sampled without the overhead and complexity of a ring buffer or an analog delay line. As manufactured, the stimulus pulse amplitude could only be set manually using a potentiometer. In addition to requiring manual amplitude adjustment, this single turn potentiometer had a limited resolution -approximately 1% of the full range (270° sweep from 0 - 100% with 20 marked divisions).

Brown (1973), while not addressing the aspect of computer control, obtained a substantial improvement in resolution by substituting a ten-turn potentiometer for the standard one. For this project, a software selectable pulse amplitude was made possible by adding the circuit shown

in Figure 3-2 to the stimulator. With this modification, the output pulse amplitude was directly proportional to the voltage applied at the control input connector. Connecting a second D-A converter output to this control input resulted in the desired full range, linear control of the stimulator with a resolution of 0.05% (50mV). The resolution of the stimulator control was apparently more than adequate considering the overlap and range of the MU thresholds as discussed in Chapter 5. The operator was directed from the program to manually set the "OUTPUT MULTIPLIER" control located on the stimulator face-plate. This was only a minor deficiency, as the position of the control needed to be changed only once or twice during a test.

Figure 3-2 Stimulator Circuit Modifications



Note: S1 and J1 are mounted on the front panel. The remainder of the circuit is on a printed circuit board inside the stimulator.

3.1.4. Signal Processing Equipment

The front end processor was designed and built by the staff of the Biomedical Engineering Department at Chedoke Hospital (Hamilton). The input amplifier consisted of the standard three operational amplifier instrumentation configuration. This amplifier provided a voltage gain of 1020, a common mode rejection ratio of 92 db, a high-pass cutoff at 5 Hz and approximately 10 uV of noise referred to the input. The input amplifier was connected to the rest of the analog system by a long cable. This permitted it to be positioned near the subjects' hand thereby minimizing the length of the low level signal path. The front-end processor provided a low-pass cutoff frequency of 1 kHz, and a variable gain control which was set by the operator. The input amplifier was electrically isolated from earth (power line ground) to minimize 60Hz noise and to reduce the possibility of dangerous currents passing through the subject should a ground fault occur.

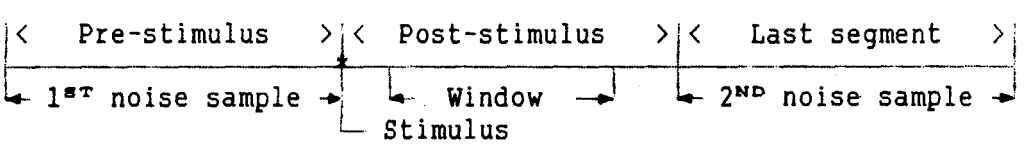
3.1.5. Data Acquisition

The stimulation and signal acquisition were performed by an assembly language program which is described in detail in the appendix. A short discussion of the program is appropriate now because the program is common to many of the discussions to follow, and it was the means of interfacing the computer to the physical world.

As noted above, a single A-D input channel of the LPS-11 was used to accept the EMG signal as amplified and filtered by the front end pro-

cessor. The data acquisition was driven by the LPS-11 real-time clock. At equally spaced intervals, an assembly langauge routine transferred the 12-bit data samples from the A/D converter to a memory array for use by the processing programs. A sampling rate in excess of the Nyquist requirement (2 kHz) was used to obtain a finer resolution in the latency measurements. The software routine set the stimulator amplitude control voltage, acquired one segment of EMG data, triggered the stimulator, then acquired two more equal length signal segments as depicted in Figure 3-3. Thus three contiguous signals were returned; a pre-stimulus signal, the signal immediately following the stimulus, and a third segment which followed the second signal. The first and third segments of data were normally used to assess the amplitude and the stationarity of the background EMG as explained later in this chapter. The segment length was chosen to ensure that the second segment would embrace the stimulus artifact and any evoked motor responses; it was this signal that was processed to obtain the data required to estimate the number of functional motor units.

Figure 3-3 Time Relationship of the 3 Data Segments



3.1.6. Graphical Display

Use of the Tektronix graphics display terminal eliminated the need for the storage oscilloscope - the display format was designed to mimic the oscilloscope display (see for example, Figure 4-4). An axis system with the origin at the screen centre was marked with one centimetre divisions, the horizontal axis indicating zero volts, the vertical axis marking the time of stimulus triggering. The horizontal and vertical scales were automatically selected by the display program, such that the saturation level of the A/D was located near the full scale of the display, and the entire signal would fit horizontally on the screen. A permanent copy of the graphics screen could be produced at any time during the processing by pressing a button on the hard copy unit. The console terminal was used to display the text necessary to permit the operator to interact with the program, or to monitor the test progression.

3.2. Development of the Protocol for the Automated Technique

An examination of the protocols used in the manual and automated implementations of the McComas technique comprises the bulk of this chapter. The technique requires the completion of a series of tasks:

a) selection of the muscle to be assessed
b) placement of the stimulation electrodes
c) placement of the recording electrodes
d) identification of the motor threshold
e) detection of a verifiable change in the EMG response
f) control of the stimulus amplitude
g) identification and rejection of noisy EMG samples
h) limiting the acquisition of distinctive EMG responses
i) measurement and recording of the evoked responses and their statistics
j) identification of the MEP response
k) measurement and recording of the MEP responses and their statistics
l) calculation of the MU count estimate
m) estimation of the confidence interval for the MU count.

These tasks will now be discussed in detail.

3.2.1. Muscle Selection

The criteria for selecting a muscle for testing were a consequence of the project's primary objective, which was to develop a method and not to investigate any particular muscle or disorder. The thenar muscle was selected for testing for many reasons. First of all, it was relatively easy to work with. Both the thenar and the median nerve which innervates it could be accessed without disturbing the subjects' clothing, and electrodes could be installed while working at a convenient height. Secondly, the thenar met the majority of the criteria discussed in Chapter 2. That the thenar is an acceptable candidate for the test is evident from the numerous studies which are based upon it (Brown, 1972, 1973; Kadrie et al., 1976; Milner-Brown and Brown, 1976; and Sica et al., 1974). Finally, none of the controversy surrounding the use of the other

commonly tested muscle, the extensor digitorum brevis, has been extended to the thenar.

3.2.2. Stimulation Electrodes

Correct stimulus electrode placement is essential to minimize cross-talk from other muscles of the hand. Figure 2-4 shows the stimulation and recording electrodes used by McComas to count the motor units in the thenar. The stimulation electrodes consist of two AgCl disks 1 cm in diameter with a 3 cm centre to centre separation. The cathodal stimulating electrode was the more distal electrode. These electrodes are positioned over the median nerve and are fastened to the skin using adhesive tape. The effect of this positioning is to limit the direct or indirect stimulation of the muscles lying higher up in the forearm or other muscles in the hand which are innervated by the ulnar nerve.

The stimuli consisted of rectangular voltage pulses 50 μS in duration (Sica et al., 1974). An excitable cell membrane has associated with it a strength duration curve which defines the amplitude of the current necessary to elicit an AP response using a given pulse width. Selection of the optimum pulse width involves consideration of factors related to tissue injury (energy dissipated per pulse, charge transfer per pulse, peak voltage), pain response and the slope of the evoked response vs. stimulus amplitude curve. The relatively short pulse width used by McComas and in this study was suitable considering the above factors. In the case of transcutaneous stimulation, the electrodes are connected to the tissue via a saline gel bridge. Because of this, tissue injury is unlikely and the duration can be selected to minimize the pain

(Crago et al. 1974). A shorter pulse width as used by McComas diminishes the pain sensation associated with the stimulation (Vodovnik et al., 1965). A short pulse width also increases the threshold difference between different diameter nerve fibres (Gorman and Mortimer, 1983). This may introduce a bias due to motor unit size in the estimation process. McComas used a monophasic pulse which resulted in a less pronounced bias than would have been evident using a biphasic pulse. The protocol used in this study conformed to the McComas methodology except that stainless steel disks coated with conductive electrode gel were used in place of AgCl disks for ease of construction.

The median nerve supplies most of the flexor forearm muscles, and is concealed by the flexor digitorum superficialis for most of its length. It continues as a large trunk down the forearm and into the hand before subdividing (Hollinshead, 1976), and can be accessed for stimulation just above the wrist. This limited, large scale branching ensures that all MUs contributed to the MEP, although the branching of individual axons within the nerve discussed in Chapter 2 is not precluded.

To check the position of the stimulating electrodes, the subject reports the region of the hand experiencing a sensation when stimulated. The median nerve consists mainly of sensory fibers from the palmar and distal portions of the dorsal surfaces of the thumb and first two and a half fingers. Therefore, a sensation towards the ulnar side of the hand indicates that the electrode should be moved in a radial direction.

In addition to the motor branch which innervates the thenar muscles, the median nerve also innervates the first two lumbricals and rarely (3-10%), the first dorsal interosseous (Hollinshead, 1976). Since

these are deep muscles, and located distal to the thenar, it is expected that the effects of any cross-talk from these muscles will be negligible. The ulnar nerve passes through the wrist as well, and poses a potential source of cross-talk effects due to inadvertent stimulation. To minimize this problem, the stimulating electrodes are positioned towards the radial side of the median nerve to make best use of what physical separation there is, and the stimuli used to obtain the MEP samples limited to values just exceeding supra-maximal.

3.2.3. Recording Electrodes

Correct surface EMG recording electrode placement is essential to ensure appropriate summation of the MUAPs of the selected muscle. The recording electrodes used by McComas were 6 cm * 0.6 cm strips of silver. The electrode attached to the positive input of the differential amplifier lay transversely across the thenar eminence (see Figure 2-4). The reference electrode was wrapped around the little finger. A lead ground electrode was placed on the dorsal side of the hand. All these electrodes were fastened to the skin using adhesive tape. The recorded signals were filtered using a band pass of 2 Hz to 10 kHz.

In this study, recording electrodes of the same size as McComas' were used. The size of the recording electrodes produces a filtering effect on the EMG signal. Because electrodes have a higher conductivity than tissue, they act as averagers giving a mean value of the potential field in contact with the electrode surface. The signal will not be modified as long as the area of the electrode is small relative to the wavelength of the signal (Plonsey, 1965). Thus, the relatively wide

(0.6 cm) electrodes used by McComas will produce a low pass filtering effect which limits the EMG signal content to approximately 1 kHz for an AP with a propagation velocity of 6 m/S. However, the filtering properties of the tissue intervening between the active fibers and the recording site also create a similar effect with band-width decreasing as the distance increases (De Luca, 1979).

The positive input electrode was placed by McComas (and also in this study) over the motor end-plate zone. Selecting muscles with a narrow endplate zone ensures that the different MUAPs would be in phase as recorded by the electrodes, and would tend to summate algebraically.

The reference electrode was positioned so as not to lie on top of active muscle which would potentially generate unwanted signals. In this study, the reference electrode was placed on the thumb around the second metacarpal. This was done to decrease the amount of active tissue between the two recording electrodes.

The ground electrode ensured that the common mode signal did not drift beyond the power supplies of the differential amplifier. McComas recommends that, where physically possible, the ground electrode be positioned between the recording electrodes and the cathodic stimulating electrode (McComas, 1977). This minimizes the stimulus artifact. In this study, the ground electrode, a 2 cm x 0.6 cm AgCl electrode, was positioned on the wrist between the stimulation and recording electrodes. This was done to alleviate problems encountered with the amplifier due to the large stimulus artifact.

In this study, the recorded EMG signal was band pass filtered from 6 Hz to 1 kHz. Using surface electrodes, no useful signal is expec-

ted above 1 kHz. The low limit was determined by the characteristics of the preamplifier used. This narrower band pass reduced the effects of baseline drift and the amount of instrumentation noise.

3.2.4. The Motor Threshold

Identification of the motor threshold stimulus is important to ensure that the first evoked response recorded is the result of a single MU, and that no smaller responses have been disregarded. To manually identify the stimulus required to excite the first MU, the nerve is stimulated with a series of pulses of increasing amplitude. The operator checks for a MUAP by examining the response to the stimuli. When a MUAP is recognized, the operator hunts for a consistent threshold by reducing and increasing the amplitude of the stimulus by small amounts in a binary search fashion. This method is advantageous in that it does not require compliant subjects or subjects with sensory perception. However, it risks missing those MUAPs which are too small to distinguish from noise.

An alternate method has been used to automate this process. This method makes use of the fact that the sensory axons are generally smaller than the motor axons, and for this reason, the sensory threshold stimulus value is slightly less than the motor threshold stimulus value. The identification of the precise motor threshold value is unimportant, because some samples of the sub-threshold signal are required. Sub-threshold signal samples are required to ensure that no small MUAPs are missed, and to estimate the stimulus artifact. The procedure used to pinpoint the sensory threshold is to gradually increase the stimulus until the subject reports a periodic tapping sensation either at the

stimulus electrodes, or in the hand being tested. As soon as the sensation is reported, the operator presses the Schmitt trigger button. This halts the stimulation and displays the response to the final stimulus pulse, that is, to the stimulus value which will be used to obtain the samples of the sub-threshold EMG response. The operator scrutinizes the display for any EMG response, and either accepts it as sub-threshold, or rejects it and ensures that the subject has understood the instructions before restarting the graded stimuli. Because the sensory threshold is sufficiently below the motor threshold in all of the subjects tested (see results, Chapter 4), it was not necessary to automate this check for a MUAP. Had it been necessary, then tests similar to those used to detect an increase in the evoked response would have been employed.

An error in the automatic identification of the threshold stimulus would not have serious consequences (such as a safety hazard or an apparently successful test producing an incorrect estimate). If the true threshold were considerably larger than the value identified, then the only cost would be the additional time required to process a slightly greater number of sub-threshold signals. If the stimulus value identified as sub-threshold were in fact supra-threshold then the evoked MUAP would have become apparent to the operator as the samples underwent ensemble averaging.

The display of the signal evoked by the threshold stimulus also provides a means of checking the analog connections: the baseline appears relatively flat, while some noise and a stimulus artifact is clearly evident as illustrated in Figure 4-4. Often, a few trials are made to identify the sensory threshold, and the average of the stimuli recorded

for the successful ones (those identified as sub-threshold) is defined as the threshold stimulus and used as the starting level during data collection for the average MUAP. In most cases, a single run to estimate the threshold stimulus is sufficiently accurate. However, where multiple estimates have been obtained, this averaging provides an added degree of robustness to the estimate.

The stimulus step size and rate are selected so that the threshold is approached reasonably slowly to prevent overshooting the motor threshold, and to allow the subject to develop a degree of confidence in the stimulation. The restriction on these parameters is that the test interval not be prolonged unnecessarily. If the maximum stimulus was reached before the Schmitt trigger was pressed, then it is likely that there was a problem with the stimulator or the subject had misunderstood the instructions. Problems are corrected before testing is restarted.

3.2.5. Detection of a Change in the Evoked Response

Detection of a valid change in the evoked response is a fundamental part of the McComas algorithm. Ballantyne and Hansen (1974a) have formalized the decision process involved as the operator views the responses appearing on the screen. The operator can:

> "(1) define the sample as a new template, or (2) incorporate the sample into the template which is [closest to it as measured by the area of the difference potential]. Or he can (3) delete the last template[, ... or,] the sample will be replaced by a new sample".

The system described in this thesis has incorporated decision criteria which automate the selection of these options. This classification of the response is the source of much of the subjectivity in the manual implementation of the McComas method. Whether done manually or automatically, this assignment requires a means of detecting an increment in the response compared with the previously evoked responses. Implicit in this is retaining the existing templates and selecting from them the template which is most like the response. These are the two tasks which Ballantyne and Hansen have assigned to their computer. The first task, maintenance of the templates, is prone to error and bias when done manually using only an oscilloscope and the operators skill, but is easily accomplished using a computer. The second item is essentially a pattern recognition task and is probably done better by man than by machine. However one of the goals of this project was to eliminate any operator dependent decisions.

The implementation developed is modelled after the manual method. When the background noise is within bounds, the evoked signal is compared with all existing templates, and the degree of match is assessed. The signal forms a new template when the best degree of match is inadequate. Where the signal matches an existing template, it is assigned to that template - the signal, with the estimate of the stimulus artifact and baseline shift removed, is used to update the existing template.

The comparison of a response with the previously formed templates is accomplished in the manual technique by a complex and loosely defined set of criteria including the relative shape, size and position of the two signals. These criteria are combined with a threshold at which the

match hypothesis is accepted or rejected. Automation entails the formalization of this heuristic procedure using statistics, or distance measures, to describe the degree of match. Using these measures, the template which "best" matches the signal can be selected. Thresholds on the distance measure statistic for this "best" match then dictate whether the signal should be categorized with that template or if the signal should form a new prototype.

The differences between a signal and the best fitting template may be due to a change in the set of MUs producing the signals or may be due to noise. If a difference between the signal and the template was caused by a single MU firing (or ceasing to fire), then the difference signal should be the MUAP of that MU (inverted if it has ceased to fire). As discussed in earlier chapters, MUAPs can vary greatly depending upon recording conditions and the nature and site of any disorder. It is desirable to design a system which is unconstrained by pre-defined MUAP shapes. The trade-off for such generality is an increased risk of falsely accepting noise as a MUAP.

Measurements which are used to describe or compare the signals are called features. The most comprehensive set of features available for comparing signals is the set of time samples. To reduce the computational time required, it is generally desirable to reduce the number of features, while minimizing the amount of discriminatory information lost because of the reduction. Andreassen (1983) evaluated the performance of a MUAP identification scheme using combinations of seven features of the MUAP, or using the time samples of the measured signal as features. The entire set of 280 time samples available to him provided the lowest

classification error rate (0.12%) and so was used in this project to classify the signals during acquisition. Andreassen found that a classification based upon peak amplitude, peak-to-peak amplitude, and area gave a low error (0.3%). These features are among those calculated by this proposed system. He found that the error rate was not improved by considering all seven features proposed (duration, peak amplitude, peak-to-peak amplitude, area, power, number of phases, and maximum slope).

Only a portion of the signal within a pre-defined window w, (Figure 3-3) is considered when calculating the degree of match. Two statistics, A and D are used to measure the degree of similarity. The statistic "D" is the square-root of the sum of the squared differences. This corresponds to the Euclidean distance in W-dimensioned space, where W is the number of samples included in the window.

$$D = \sqrt{\sum_{i=1}^{w} (S_i - T_i)^2}$$

Where S_i and T_i are the signal and template samples respectively.

The second similarity measure used is "A", the absolute area between the signal and the template under consideration:

$$A = \frac{\sum_{i=1}^{w} |S_i - T_i|}{w}$$

Any one of three conditions will prevent a signal from being assigned to the closest template :

a) $$\frac{\text{(The smallest value of D found for any template)}}{\text{(Std dev'n for the noise) * (\# samples in window)}} > \text{Limit 1}$$

b) $$\frac{\text{(The smallest value of A found for any template)}}{\text{(The area expected due to noise)}} > \text{Limit 2}$$

c) If different templates were independently selected as "best" by the two distance measures.

By normalizing with respect to the noise, the empirical limits do not have to be altered for each subject, thus decreasing the operator interaction. If any one of these three conditions is not met then the signal is not assigned to a template. This would occur due to noise or when the family of motor units producing the response changed. In the first instance, the signal would have been correctly rejected. In the second case, it was expected that the same signal would be re-evoked with the application of the same stimulus value. More will be said later about avoiding spurious template formation.

3.2.6. Control of the Stimulus Amplitude

Control of the stimulus amplitude provides the means of modulating the number of MUs contributing to the response. When the McComas method is performed manually, the stimulus is slowly increased until a change in the response is detected. The stimulus level is then decreased slightly to ensure that no response can be evoked at a stimulus level intermediate to this latest level and the levels at which the previous

responses were evoked. This also permits a check that the new response can be reproducibly evoked and therefore should form a new template. When the operator is satisfied that no intermediate response has been missed and that the new response is not a spurious result, the slow incrementation of the stimulus is resumed.

With this manual method, the stimulation rate is limited by the ability of the operator to manipulate the equipment controls - especially those of the storage oscilloscope - and to classify the evoked responses. An automated method avoids these problems. In this implementation, the automatic stimulus control mimics the manual procedure. The stimulus is manipulated in an attempt to acquire a minimum number of signal members for each template, and to ensure that no intermediate responses are missed. The stimulus is slowly incremented from the previously determined sub-threshold value. The step size is small enough to prevent skipping of intermediate MU thresholds, but large enough to minimize the time required to reach the stimulus threshold of the next recruited MU without acquiring too many samples of each template. An appropriate compromise is established by specifying a reasonably small step-size and then increasing it by a factor of three after a minimum number of responses have been assigned to the current template. This increased step size may result in an intermediate response threshold being ignored. Therefore, after starting a new template, the graded stimuli restart from a value below that which evoked the new template.

When a pre-specified number of templates have emerged, the number of members forming each template is checked. If this number is less than a minimum, the stimulus is set to a value which is expected to evoke

additional members. The stimuli used to fill a particular template are values randomly picked from within the range which previously evoked a response assigned to that template. Selecting this range of stimulus values rather than simply using the average stimulus risked evoking responses which are assigned to other templates. However, this wide range of stimulus values is also likely to evoke any intermediate responses which were missed as the stimulus was increased. In addition, responses are recruited which can be tested for correlation with the stimulus amplitude.

3.2.7. Effects of Noise

A concern identified during the development of the automated system was the effects of random noise on test performance. In particular, noise can cause errors in the decision processes, resulting in the formation of spurious templates or valid templates being overlooked. The noise encountered during signal acquisition came from many sources, but with low amplitude resulting in an adequate signal to noise ratio (see Chapter 4). The instrumentation noise is small, and 60 Hz interference all but eliminated by the high common mode rejection of the pre-amplifier. The quantization noise is negligible (typically less than 300 nV) due to the large analog gain and the 12 bit resolution of the A/D converter. Motion artifact can be controlled by physically restraining the subject's arm, the electrodes and their connections. Further reductions of noise due to cable motion and 60 Hz pick-up is obtained by using very short leads between the electrodes and the input amplifier.

Another noise source which disturbed the test was EMG activity from either the muscle under test or neighboring muscles. This ongoing EMG is usually caused by the subject's difficulty in maintaining a relaxed state in a muscle group for an extended period of time.

A positive feedback condition was identified which caused the subject needless anxiety. The subject would become tense and increase the level of tonic contraction. This EMG activity would cause continuous rejection of the signal and re-stimulation of the nerve. The uninterrupted train of stimuli would make the subject even more tense. This situation is easily prevented by enabling a non-zero stimulus only when the noise had been within bounds during the previous signal acquisition.

The stimulus artifact, discussed in more detail later in this section, was yet another source of unwanted signal. It is important to reject signals with excessive noise because these will cause the formation of new templates. Four measures were implemented to prevent this. First of all, a signal is rejected and the subject re-stimulated if excessive noise is detected. Secondly, an estimate of the stimulus artifact is removed before performing the template matching. Thirdly, a rejection of all existing templates has to consistently occur at a stimulus level before a new template is established. Finally, should a template be falsely created despite these precautions, it can be eliminated later in the processing.

The samples of EMG taken before the stimulus and after the evoked potential was complete provides the following measures of the background noise:

Baseline Drift

Excessive DC drift of the signal due to settling of the preamplifier, movement of the electrodes etc. can be detected as a large area under the rectified signal curve. A straightforward method of detecting a non-stationarity in the mean is to compare the difference between the means of the two periods with the variance of the noise. That is, the signal was rejected if

$$\frac{|\ \text{Mean1} - \text{Mean2}\ |}{(\text{Noise Std dev})} > \text{Limit 3}$$

With :
- Noise Std dev defined as the standard deviation of the rectified noise samples in the 1ST noise period.
- Limit 3 defined as an empirical value which yielded an acceptable performance for the subjects tested.
- Mean1, Mean2 defined as the averages of the samples in the 1ST and 2ND noise periods.

A/D Saturation

Distortion of the signal due to clipping by the A/D converter will cause an erroneous mismatch condition when the signal is compared with existing templates. Alternately, increments in a response can be missed if templates are formed from signals which have undergone clipping. Input voltages exceeding the $\pm$5V input range of the A/D converter are detected by checking for one of two conditions: either the mean of the pre-stimulus epoch is too close to the $\pm$5 Volt limit; or a sample of the signal within the pre-defined signal window reaches one of these limits. Thus, for a 12-bit A/D converter producing a two's offset output in the range of 0 - 4095, the acceptance conditions are:

$$(1/3 * 4095 \leq \text{Mean1} \leq 2/3 * 4095) \quad \text{and,}$$

$$1 \leq \text{Sample} \leq 4094 \text{ , for all signal samples in the processing window.}$$

The first restriction is required to prevent signal acquisition where the baseline has drifted due to a problem with the electrodes or the input amplifier. This restriction will also detect problems similar to those designated as "drift" above, but which occur over a longer time span.

Noise Amplitude

If the noise level in either the pre-stimulus or the third signal epoch is in excess of empirically determined bounds, the response is rejected. That is,

reject if (Std dev'n of noise segment samples) > Limit 4
or, (Area under rectified noise signal) > Limit 5

The stimulus artifact is another form of unwanted signal in that it does not provide any information regarding the muscle being tested. The artifact is removed by subtraction of a scaled version of the sub-threshold signal which is presumed to consist of noise, including the stimulus artifact, but no EMG signal correlated to the time of stimulus. The first template is used to record an ensemble average of the sub-threshold signal samples, thereby removing all uncorrelated noise contributions, and leaving an estimate of the stimulus artifact. A sufficiently large number of sub-threshold responses are therefore collected.

It was assumed that the stimulus artifact increases linearly with the stimulus intensity. The artifact for any stimulus value is therefore estimated by scaling the signal averaged in the first template. This permitted the removal of the artifact from signals obtained at any stimulus by subtracting the estimate from the signal. This technique is employed to minimize the influence of the stimulus artifact upon the features and statistics, and to display signals on the Tektronix screen:

$$\text{Display} = \text{Signal} - \frac{\text{Present Stimulus} * \text{Template(1)}}{\text{(Ave. of stimuli for Template \#1)}}$$

Another method of minimizing the influence of the stimulus artifact on the statistics calculated for the signals is to consider for processing only those samples which lie within a more restricted time window. A scheme used by Ballantyne and Hansen (1974a) allowed a variable delay from the stimulus to the start of acquisition. This permitted a range of peripheral latencies and a variable duration of sampling (to 17 mS) to accommodate the duration of the longest response. The system developed here also has a variable length sampling window (see Figure 3-3) combined with a variable starting time relative to the stimulus. However, this system also has the advantage of displaying the stimulus artifact, thereby providing a precise time reference. It also displays a segment of the EMG occurring after the response which permits a checking that the evoked response does not extend beyond the boundaries of the window. The time from the application of the stimulus to the start of the window is selected to minimize the artifact content. The window width is selected to ensure that the evoked potentials are complete and the window contains only short intervals of baseline.

3.2.8. Control of Template Formation

As in the case of the manual system, a new template is formed when a response can be consistently evoked which does not match any previous response. While it is important not to miss the occurrence of a small or infrequent response, it is also desirable to prevent the proliferation of templates caused by random noise. This is accomplished by specifying that an empirically determined number of signals (typically 2) not matching any of the existing templates occur consecutively at a constant stimulus level before their ensemble average is accepted as a new template.

To identify a valid response, which should form a new template, an unassigned response is copied into an array and the subject re-stimulated with the same stimulus value. If the responses at this stimulus level consistently match the average signal stored in the array, without matching the other existing templates, then the ensemble average of these responses forms the new template.

Despite the precautions employed to prevent the formation of spurious templates, a number of instances occur where the noise meets the required conditions and a template is erroneously formed. To address this situation, a minimum number of members is necessary to retain a template. If numerous (typically 25) attempts to recruit further members of a particular template fail to evoke a few (typically 3) members, that template is discarded. This coincides with the situation encountered during manual implementation and described by Ballantyne and Hansen

(1974a) who permit the operator to discard a template "if the potential contained therein is thought to be spurious".

It was necessary to develop a criterion which, when satisfied, will halt the acquisition of data during the search for discrete increments in the evoked response. When testing manually, the operator stops looking for additional increments in the response when the mechanism for discriminating is frustrated. This happens when, at a constant stimulus, more distinctive responses occur than can be remembered. A similar criterion was incorporated in the automatic counting method. When the memory available to store the templates is exhausted the acquisition of new data stops.

Since it is desirable that most of the templates be the ensemble average of a statistically large number of signals, the stimulus is manipulated to fill the existing templates while a sufficient number of template positions remained vacant. These vacant positions are required to accommodate new templates generated during the acquisition of signals augmenting the existing templates. The system developed features 17^2 positions for templates, versus the 15 available in Ballantyne and Hansen's system.

As discussed above, where a template was established but only a few additional responses can be evoked to match it, the template is eliminated thereby freeing the memory space for use by a valid template. The increase in the number of template positions and the recycling of the

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[1] These do not include positions used for the sub-threshold samples, for the MEP samples (Ballantyne & Hansen), or temporary storage arrays.

memory space permits more MUs to be incorporated in the calculation of the average MUAP, resulting in a more exact estimate.

3.2.9. Estimation of the Maximum Evoked Potential

A method was developed to identify the stimulus level required to evoke the MEP response. The automatic method used to establish the MEP signal is the same as that of the manual method. The stimulus level is increased until no further increase is perceived in the evoked response. The algorithm requires the control of the stimulus and a criterion for detecting an increase in the response.

The step size used to increment the stimulus is bounded by two constraints. Too small a step size (such as that used to obtain the first few responses near threshold) will result in a long and painful sweep up to the level required to recruit all of the MUs. In addition, a small step size will increase the possibility of falsely identifying a local plateau in the response/stimulus function as the MEP. On the other hand, too large a step size risks overshooting the stimulus level required to barely evoke the MEP. The consequences of this will be excessive pain due to stimulation, and stimulation of adjacent nerves resulting in cross talk from other muscles. The step size used is a multiple (software selectable) of the increment used to grade the stimulus near the motor threshold.

The stimulus levels required to obtain the MEP samples often exceed 200 V, which requires that the "*4" control setting be selected. At this setting, it is possible to apply pulses of 400 V to the subject. This is an uncomfortable level, but not dangerous, or even painful when

applied as a single pulse. However, the sensation of pain was found to increase with an increase in the stimulation rate. For this reason, the stimulation was designed to be semi-automatic, requiring a carriage return to enable each stimuli. This minimizes the possibility of applying a long train of high voltage stimuli during the search for the MEP threshold.

As the stimulus is incremented, an increase in the response is detected using two distance measures, the area between the present and previous responses, and the average increase in the corresponding time samples of the two responses. When the value of the area measure shows that the response has changed by less than a pre-specified fraction of the total response, and that this change is an increase, the response is designated to be the MEP. That is, when both of the following conditions were satisfied the response was termed the MEP:

$$\frac{\text{(Change in area from last response)}}{\text{(Total area of latest response)}} < \text{Limit 6}$$

$$\text{(Average change in time samples)} \geq 0$$

If the value selected for Limit 6 is too small, then the deviation expected due to noise and stimulus artifact will result in the use of an excessively high stimulus value to acquire the MEP samples. On the other hand, if the value for Limit 6 is too large, the stimulus amplitude might have been inadequate to evoke the MEP, resulting in an underestimate in the MU count. To detect this second problem, the stimulus values used to evoke the samples of the MEP are varied randomly about the stimulus value which evoked the response first identified as the MEP. The coefficient of

correlation is calculated to provide a measure of the relation between the stimulus amplitude and the area or peak of the evoked response.

Noise is not as much of a problem while acquiring the MEP data as when acquiring data with which to estimate the average MUAP. This is because the final motor unit number estimate is much less sensitive to measurement errors in the MEP. A 5% variation in the peak or area of the MEP response would have resulted in only a 5% variation in the estimate of the number of viable MUs. A similar variation in the responses recorded to estimate the average MUAP could cause the formation of many false templates and failure of the algorithm. Nevertheless, the interference due to noise is minimized during the acquisition of the MEP signal samples.

Measures and thresholds employed are similar to those discussed previously, except that the slow decay from the large stimulus used to evoke the MEP is cause for ignoring the second noise epoch samples. Hence, the test for stationarity of the mean was not implemented for these samples. The lower analog gain (typically *500) needed to prevent saturation of the A/D converter results in a decreased resolution in the amplitude measurement. The quantization error (approximately 5 uV) is of the same order of magnitude as the instrumentation noise. Therefore, a lower limit on the threshold was implemented to ensure that no signal is rejected by a noise measurement which could be explained solely by this quantization effect.

3.2.10. Estimation of the Number of Motor Units

A total of six methods are used to calculate estimates of the number of viable MUs in the muscle tested. McComas used peak as the defining feature in his calculations for obtaining the count estimate, while others have used the absolute area of the potential (Ballantyne and Hansen, 1974a). Three basic methods are employed, first using the peak amplitude and then the area as the features applied in the calculations. This permitted a comparison of the two features used to calculate the estimate. All three of the basic methods require that the responses be ranked according to the feature values. An implicit assumption was that the larger responses are composed of more MUAPs.

Two schemes for obtaining the feature (either area or peak) for the average MUAP result in two of the basic estimation methods. The first method is identical to the one used manually. The selected feature of the largest of the templates evoked at low stimulus amplitudes is divided by the total number of templates (excluding the first template which represents the stimulus artifact). Thus, if there were 11 templates, then the feature of the largest is divided by 10 to obtain the feature of the "average MUAP". The second method is to decompose the compound responses to extract the individual MUAPs, calculate the features for these MUAPs, then take the "average of the features" of the MUAPs. The two estimates differ because of the effects of non-linear summation.

For both methods, the average feature calculated is divided into

the corresponding feature determined for the MEP. This results in the count estimate:

$$\text{Count by method A} = \frac{(\text{MEP feature}) * (\text{number of templates} - 1)}{(\text{feature for largest template})}$$

$$\text{Count by method B} = \frac{(\text{MEP feature}) * (\text{number of templates} - 1)}{\sum_{i=1}^{n} (\text{feature of } i^{th} \text{ MUAP extracted})}$$

The third method was developed to make use of the information content of all of the responses evoked, not simply the largest response, and has the further advantage of providing a framework for testing alternate, non-linear models for the summation of the MUAPs. By using regression analysis on the response feature versus number of contributing MUs data, an estimate of the number of MUs contributing to the MEP is obtained by extrapolation :

$$\text{Count by method C} = \alpha + \beta * (\text{MEP feature})$$

where the linear regression parameters "α" and "β" are calculated from the N-1 compound response features, the X_i's, and the number of units

contributing to the responses, the Y_i's, as follows :

Regression parameters:

$$\beta = \frac{\sum_{i=1}^{N-1} (X_i - \bar{X}) * (Y_i - \bar{Y})}{\sum_{i=1}^{N-1} (X_i - \bar{X})^2}$$

$$\alpha = \bar{Y} - \beta * \bar{X}$$

Once the evoked EMG responses are ordered, they are decomposed into the MUAPs which formed them as was done by Ballantyne & Hansen (1974b) to obtain data on latencies, amplitudes, durations, and areas of the MUAPs. The MUAPs are recovered from the compound potentials by subtracting the lower ranked template from each template. The MUAPs are displayed on the graphics screen and the operator is permitted to discard any of the templates which he judges to be identical to a previously accepted template - that is, where the differential signal does not appear to be a MUAP. This provides a means of recovering from the accidental creation of a template when there had been no bona fide change in the response. The ensemble average is calculated for the responses which were accepted as valid. This signal normally was indistinguishable from a scaled copy of the largest template of the evoked responses.

Associated with each estimate of the number of MUs is a confidence interval obtained by considering only the mathematical aspects of the test calculations. The other procedural and physiological causes of inaccuracies and variations fundamental to the test have been discussed in the earlier chapters, and attempts to quantify them must come from

outside the test - either from alternate estimation methods, or from studies involving multiple trials or subjects.

To obtain confidence intervals for the three methods presented above, the number of MUs sampled from the entire population of MUs must be considered. Also, the measurement accuracy of the data acquired will have a bearing upon the accuracy of the calculations. For the estimates obtained by method A and method B, the variance in the count is estimated using uncertainty analysis (Holman, 1978):

$$s_C = \sqrt{\left(\frac{\delta C}{\delta M} * s_M\right)^2 + \left(\frac{\delta C}{\delta A} * s_A\right)^2}$$

Where : C refers to the Count estimate of the MUs
M " " the MEP signal feature
A " " the Average MUAP signal feature

Which, for $C = M/A$, reduces to :

$$s_C = \frac{1}{A} * \sqrt{s_M^2 + C^2 * s_A^2}$$

The variances for the MEP and the average MUAP features are estimated from the data corresponding to the multiple samples of these responses.

The estimate of the variance for the count from method C was the

standard error of forecast for the linear regression which is given by the following expression :

$$t_{0.05} * \sqrt{\left(1 + \frac{1}{N} + \frac{(X_0 - \bar{x})^2}{\sum_{i=1}^{N} (X_i - \bar{x})^2}\right) * \frac{\sum_{i=1}^{N} (Y_i - \hat{y})^2}{(N - 2)}}$$

With

$\bar{x}$	:	average of the features of the templates
X_0	:	value of the feature for the MEP
X_i	:	value of the feature for the i^{TH} template
$\hat{y}$	:	estimate of number of MUs from method C
Y_i	:	number of MUs forming the i^{TH} template
N	:	number of MUs (= number of templates - 1)
$t_{0.05}$	:	t-distribution value for N-2 degrees of freedom

Thus, six estimates with associated confidence intervals are presented at the completion of the testing. In addition to the textual presentation of the results, the graphics terminal displays the MEP signal simultaneously with the corresponding MUAP average signal scaled by the estimate of the number of MUs. The discrepancy in the two shapes provides an indication of the representative nature of the MUAPs used in the estimation algorithm, and of the effects of nonlinear summation of the signals. As well, the correlation of the features of the MUAPs with their order of recruitment is calculated to permit an assessment of the degree of bias in the method of recruitment of the MUs.

This chapter has described the development of the algorithm used in the automated motor unit counting system. Further details of the algorithms developed for the test system may be found in the appendix.

CHAPTER 4.

PERFORMANCE OF THE ESTIMATION SYSTEM

In the previous chapters, the theory and design of an automatic MU counting system were described. In this chapter, the results obtained during tests of this system are presented. The results are interpreted in light of the background discussions.

The purpose of these tests was to demonstrate the operation of the system using real data obtained from human subjects. This was a more stringent test of the system than would have been achieved using mathematically-modeled data. This chapter presents a qualitative discussion of the results obtained from tests of the automated motor unit counting system.

4.1. System Testing Procedure

4.1.1. Selection of Subjects

A group of students and staff members were recruited to act as subjects. None of the volunteers had a history of nervous disorders. One subject underwent a series of six runs. The remaining eight subjects each underwent the procedure once.

4.1.2. Algorithm and Parameters Used

The algorithm used for all subjects was identical to the algorithm described in Chapter 3. The parameters used in the program are given in Table 4-1. The operator did not have to adjust any of the parameters to enable the system to accommodate a particular subject.

Table 4-1: Variables and Parameters Used During Test

Variable	Value	Parameter Description
ZERO	2210	Control value to stimulator for 0 V.
PREAMP	1020.0	Gain of pre-amplifier.
MXSMPL	180	Signal Storage array size.
ST(1)	4.0	Limit 5 (Chapter 3).
ST(2)	4.0	Limit 5 (Chapter 3).
ST(3)	3.0	Limit 4 (Chapter 3).
ST(4)	3.0	Limit 4 (Chapter 3).
ST(5)	4.0	Limit 2 (Chapter 3).
ST(6)	1.0	Limit 1 (Chapter 3).
ST(7)	3.0	Limit 3 (Chapter 3).
ST(8)	2.0	# of consecutive responses before new template.
ST(9)	25.0	Target # of members per template.
ST(10)	3.0	Minimum # of responses req'd to form template.
ST(11)	0.05	Limit 6 (Chapter 3).
ST(12)	25.0	Stimulus increment factor during MEP.FOR.
ST(13)	25.0	Number of MEP responses.
SRATE	5000	Sample rate (Hz).
NN	160	Number of signal samples.
GAIN	2.0	Amplifier gain.
PAUSE	500	Additional inter-sample time (ms).
INCR	3	Stimulus size increment value.
SETTLE	20	Samples between stimulus and window start.
WINDOW	100	Samples in window.

The 5000 Hz sample rate provided an inter-sample interval of 200 µs. Thus, the 160 samples captured 32 ms in each of the three signal epochs described in Chapter 3. The window "W" used for template processing extended from 4 to 24 ms after application of the stimulus.

4.1.3. Test Protocol

In each run, operator activities were minimized by providing appropriate defaults from menus. The following activities were required of the operator:

1. The equipment was connected as described in Chapter 3.
2. The operator explained the test to the subject.
3. The stimulation and recording electrodes were fastened to the subject as described in Chapters 2 and 3.
4. The operator turned on the stimulator and ran the program INIT.FOR by typing "run init".
5. The operator was prompted to set the stimulator output multiplier control to the *1 position.
6. The program prompted the operator for a descriptive title to be used to identify the output for that test.
7. The operator selected the default run parameters in response to prompts.
8. After instructing the subject to relax his hand muscles, the operator pressed the return key to start stimulation. When the subject reported feeling a pulsing sensation from the region of the thenar eminence, the operator pressed the Schmitt trigger button to stop stimulation. If the operator was satisfied that the subject had been relaxed and had reported the sensation promptly, then he pressed "Q" to quit the INIT.FOR program. If not, the operator pressed "R" to repeat the stimulation sequence.
9. Having completed INIT.FOR, the operator then ran the program called AVMUAP.FOR by typing "run avmuap".
10. The operator was prompted to check that the stimulator output multiplier control was set to the *1 position.
11. The operator ensured that the subject was relaxed and comfortable.
12. The operator initiated data acquisition by pressing the return key to select "Continue" from a menu of options. Stimulation and acquisition of data proceeded and continued

automatically with no operator intervention except in the following circumstances:

a) If the subject was not relaxed, the program indicated that it was rejecting the signals due to excessive noise. The operator instructed the subject to relax.

b) For subjects with higher motor thresholds, the operator was prompted to increase the stimulator multiplier output control.

c) If the operator noticed that persistent alternation was occurring, the operator would press the Schmitt trigger button and enter "Q" to quit data acquisition.

d) When the system persistently rejected signals due to excessive noise in the third signal segment due to the H-reflexes, the operator would press the Schmitt trigger button and enter "H" to disable the noise calculations for the third signal segment. The operator then pressed the return key to continue testing.

e) When the system had completed stimulation and data acquisition, each template was displayed on the graphics terminal in response to the operator pressing the return key.

13. Next, the operator ran "MAXEP.FOR" by typing "run maxep".
14. The operator was prompted to set the stimulator multiplier output control to the *4 position.
15. The operator selected a lower gain setting (*0.2) and entered this value in response to the program's prompt.
16. The operator instructed the subject that the stimulation amplitude would be increased and that stimulation would be stopped anytime the subject felt that the stimuli were too uncomfortable.
17. The operator was prompted to press return before each stimulus pulse.
18. When the data acquisition was completed, the operator disconnected the stimulator and removed the electrodes from the subject.

19. Finally, the operator typed "run proces" to execute the program PROCES.FOR. This program paused after each display of graphical information. The operator pressed the return key to continue through to completion. The average total time required for the entire data acquisition procedure was approximately 25 minutes.

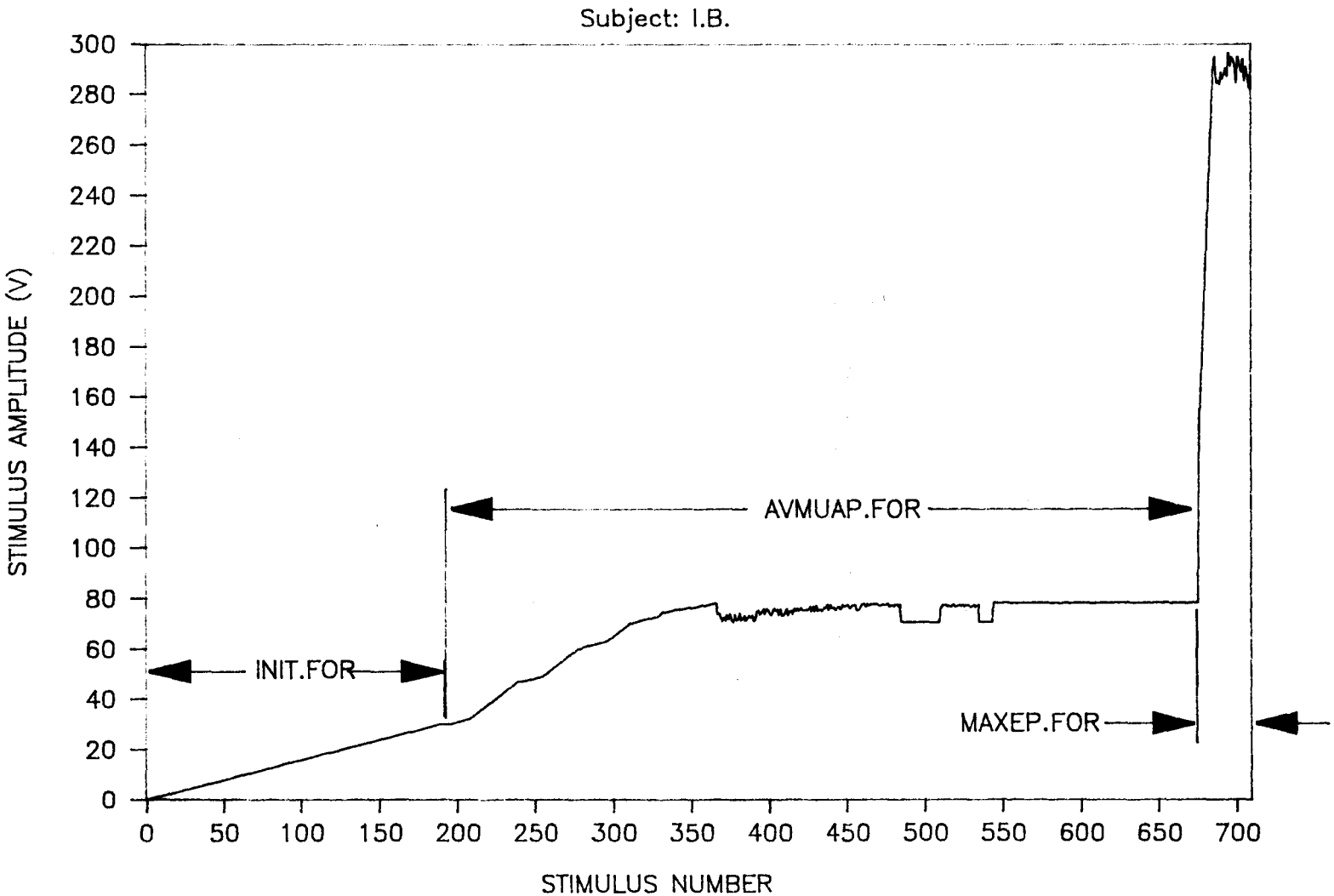
The operator activities described above required a minimum of judgment or training except in the application of the electrodes and a familiarity with the above procedure. The program was written to be user friendly by providing menus, defaults and input filtering for bad values and some limited error diagnostics on disk file use. The operator selected the defaults whenever prompted by the program. These branch points were incorporated in the program to permit a researcher greater control over the test progress and output displays.

The data acquired during these tests are presented by first discussing the program operation during a typical run. Typical output generated by the program is then displayed and discussed. Other features of the program and results of the tests are also examined.

4.2. Typical Test Sequence

A typical test sequence (subject IB) is illustrated in Figure 4-1. This plot is useful in illustrating the progress of the test. The X-axis is not time, both because the inter-stimulus interval was not constant, and because many samples were discarded due to noise, drift, etc..

Figure 4–1 **Stimulus Sequence**



The first 190 stimuli were applied by INIT.FOR in order to obtain the estimate of the sensory threshold. The stimulus was incremented in steps of approximately 0.15 V up from 0 to 30 V at which point the subject indicated that he could feel a sensation from the region of the thenar, and which recurred at the stimulus rate (approximately 5 Hz). This estimation of the sensory threshold was obtained in less than a minute.

The stimuli numbered from 697 through 731 evoked responses used to find and obtain an estimate of the MEP. Twenty five responses were obtained with amplitudes ranging between 282 - 296 V, but the size of the responses was not correlated with the stimulus amplitude (r for area vs stimulus amplitude was 0.378, N.S. at 0.05 level). This is an indication that the signal evoked was indeed the MEP.

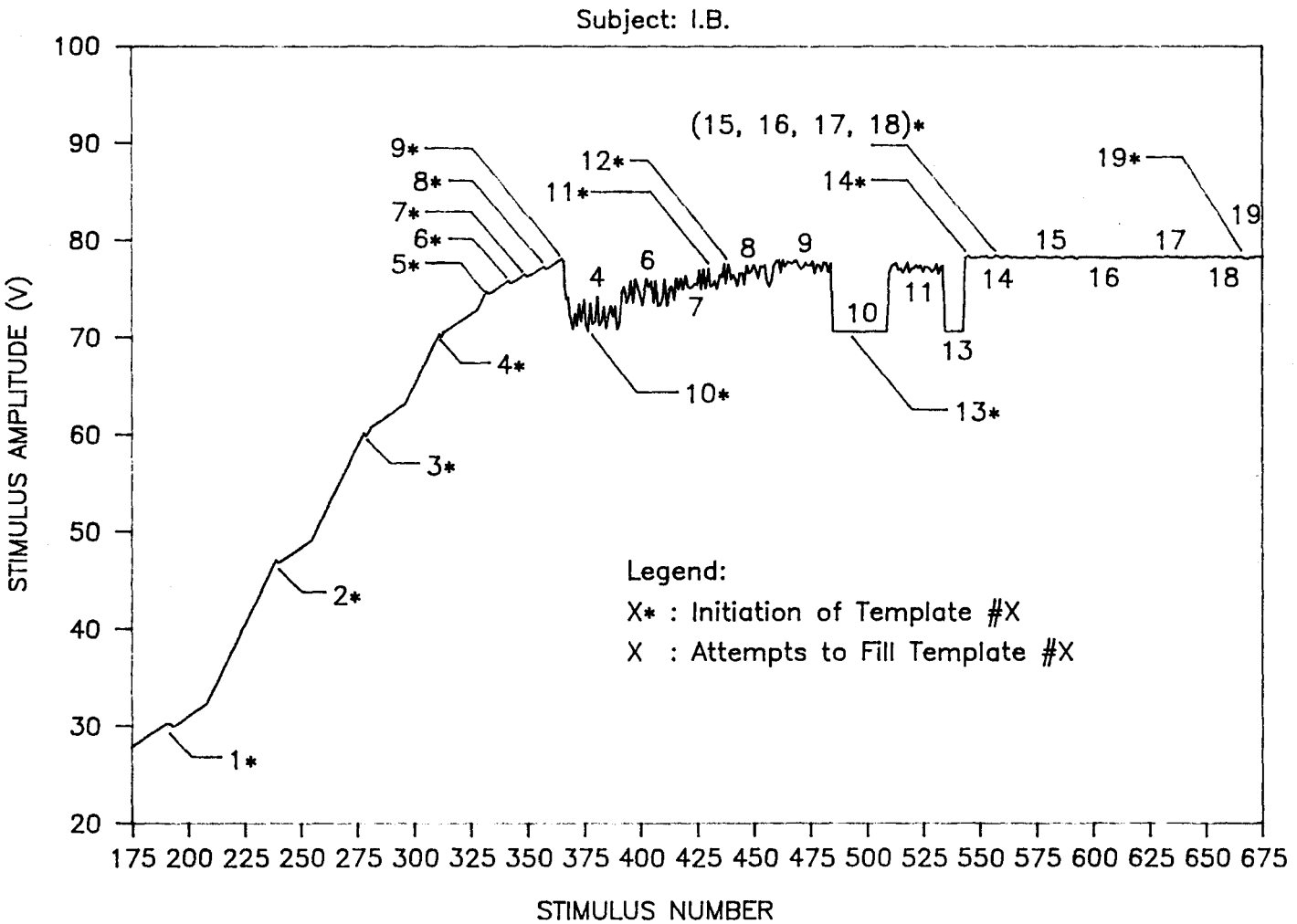
The central portion of Figure 4-1 is enlarged in Figure 4-2 to permit an expanded scale and labelling to identify the activity during execution of AVMUAP.FOR. Initially, the stimulus rate was approximately 0.5-1 Hz, the responses evoked as the stimulus was incremented up from the sensory threshold being relegated to template #1, representing the sub-threshold response. Seventeen responses were recorded without evoking any signals requiring the initiation of a second template. The stimulus increment step size increased by a factor of 3, and 30 additional responses were similarly evoked. A second template was initiated when the criteria for forming a new template were met, and the step-wise incrementing of the stimulus was restarted from an amplitude slightly below that which evoked the new template. This point is indicated on Figure 4-2

as "2*". The process of evoking members and assigning them to existing templates and starting new templates when required was repeated for a total of 9 templates. A series of new templates (#5 through #9) were initiated all within a small range of stimulus values. When 9 templates in total had been initiated, a series of 25 attempts were made to evoke responses which would be assigned to template #4. The stimulus amplitude was set to a random value within the range of values which had previously evoked the response assigned to that template.

While recruiting members of template #4, a new template (10* in Figure 4-2) was established. Similarly, the subject was stimulated with stimulus amplitudes intended to evoke additional members to templates #6 through #13. In this process, new templates were established (11*, 12*, 13*) and template #10 was discarded due to failure to evoke additional matching responses.

With all existing templates reassessed, the stimulus amplitude began to increase from the level where orderly incrementing had stopped. Templates 14* through 18* were initially established and attempts to recruit 25 members for these templates began. This processing required that two additional templates be established. Insufficient memory space prevented the establishment of the second (#20) and execution of AVMUAP.FOR halted normally.

Figure 4–2 Stimulus Sequence During AVMUAP.FOR



A total of 507 evoked responses were assigned to templates, with 151 signals rejected due to background noise or failure to evoke a repeatable response. The time required for data acquisition was less than 25 minutes.

A total of 16 supra-threshold responses were identified and stored. The estimates of the number of MU's along with interpretations of these estimates are presented later in this chapter.

4.3. Graphic Screen Displays

Various signals were displayed on the graphics screen during program execution. This section presents typical displays obtained during the test runs. The formats of the displays are presented here, while the interpretations of the signals are reserved for later sections of this chapter.

Figure 4-3 shows the graphic screen display after execution of INIT.FOR. The signal displayed is the last signal sampled before pressing the Schmitt trigger. The legend of the display provides scale information, test identification and a summary of the sensory threshold estimate.

Figure 4-3 Display Following INIT.FOR Completion

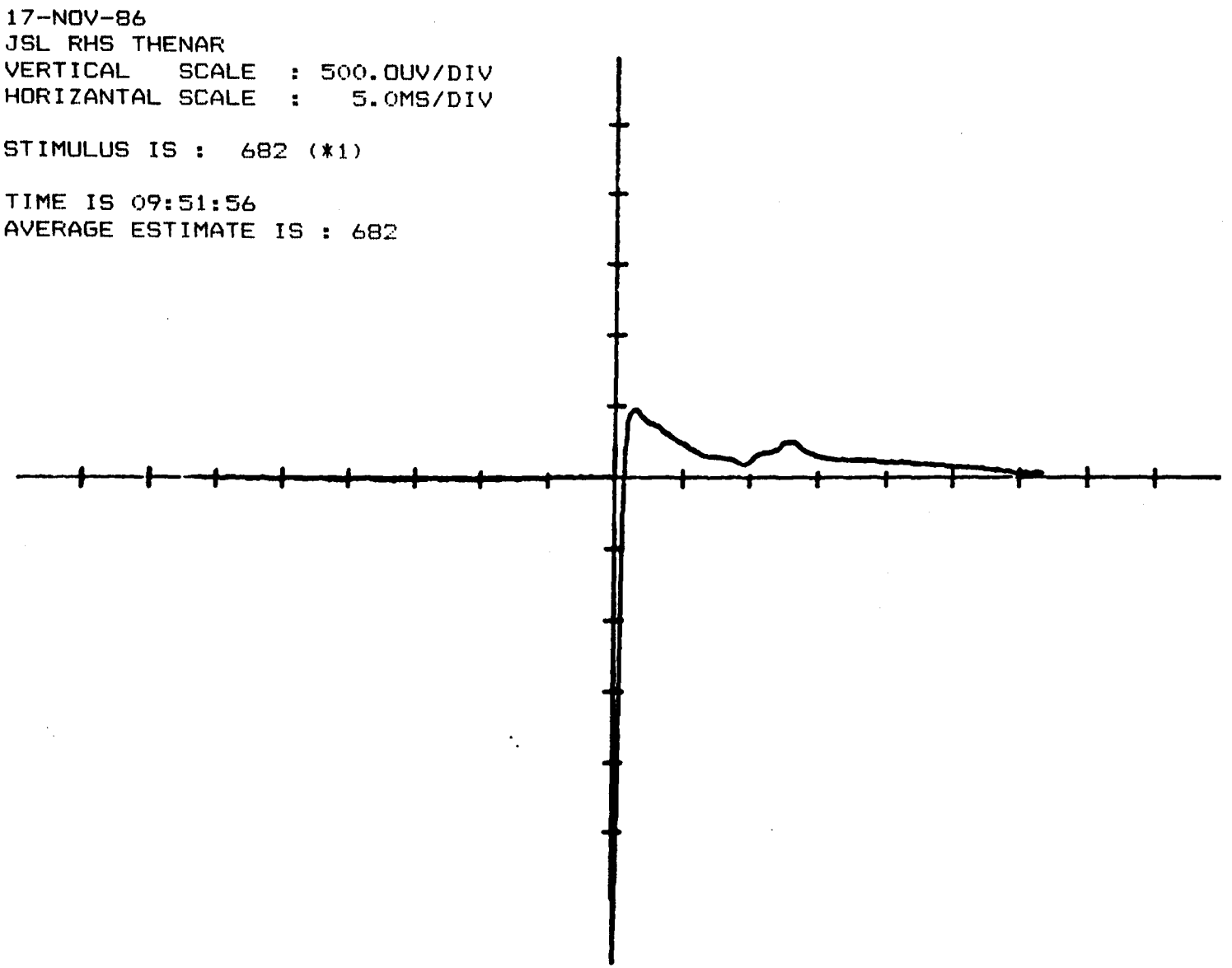


Figure 4-4 Display of Evoked Responses

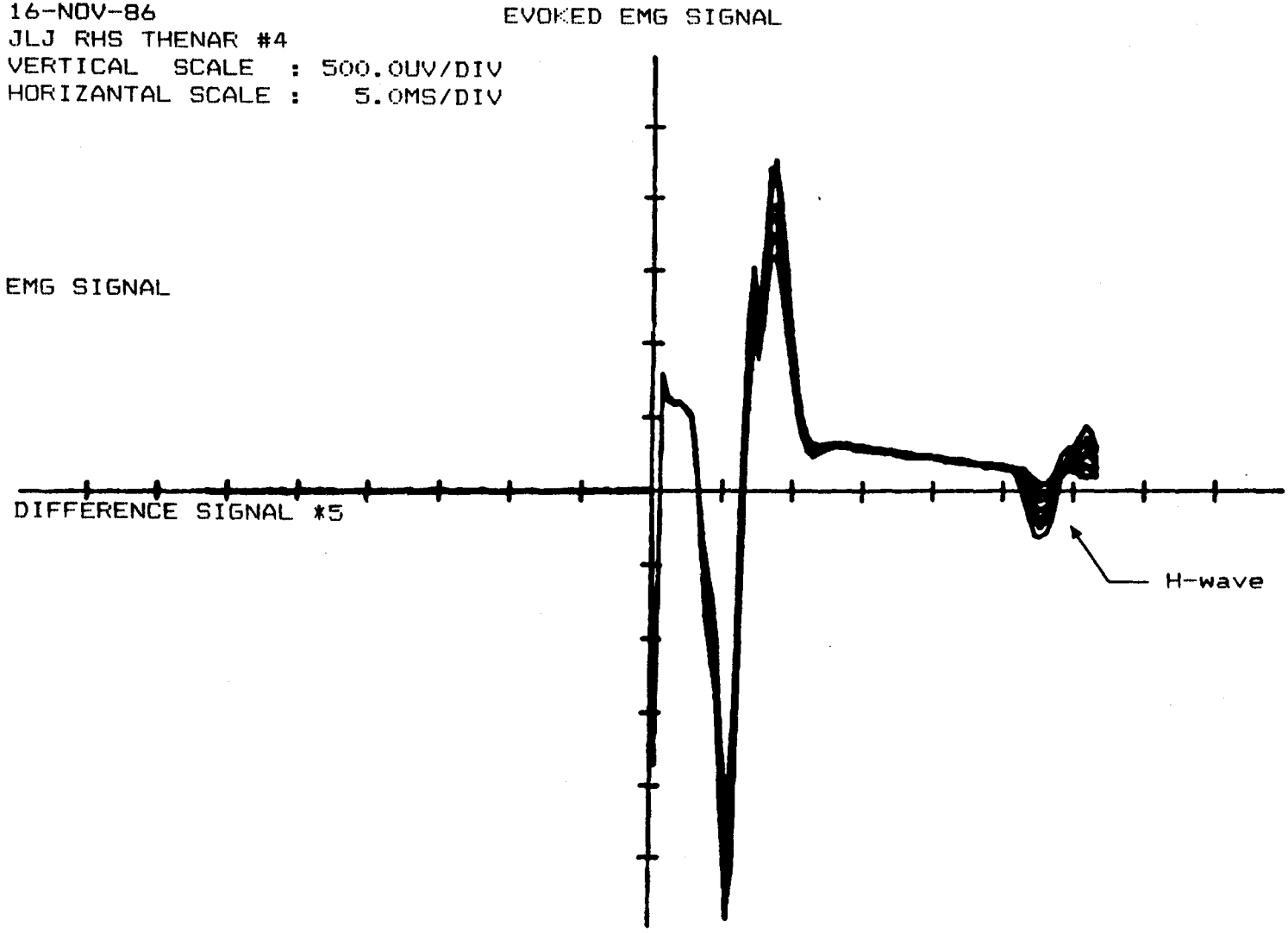


Figure 4-5 Display of Difference Signal

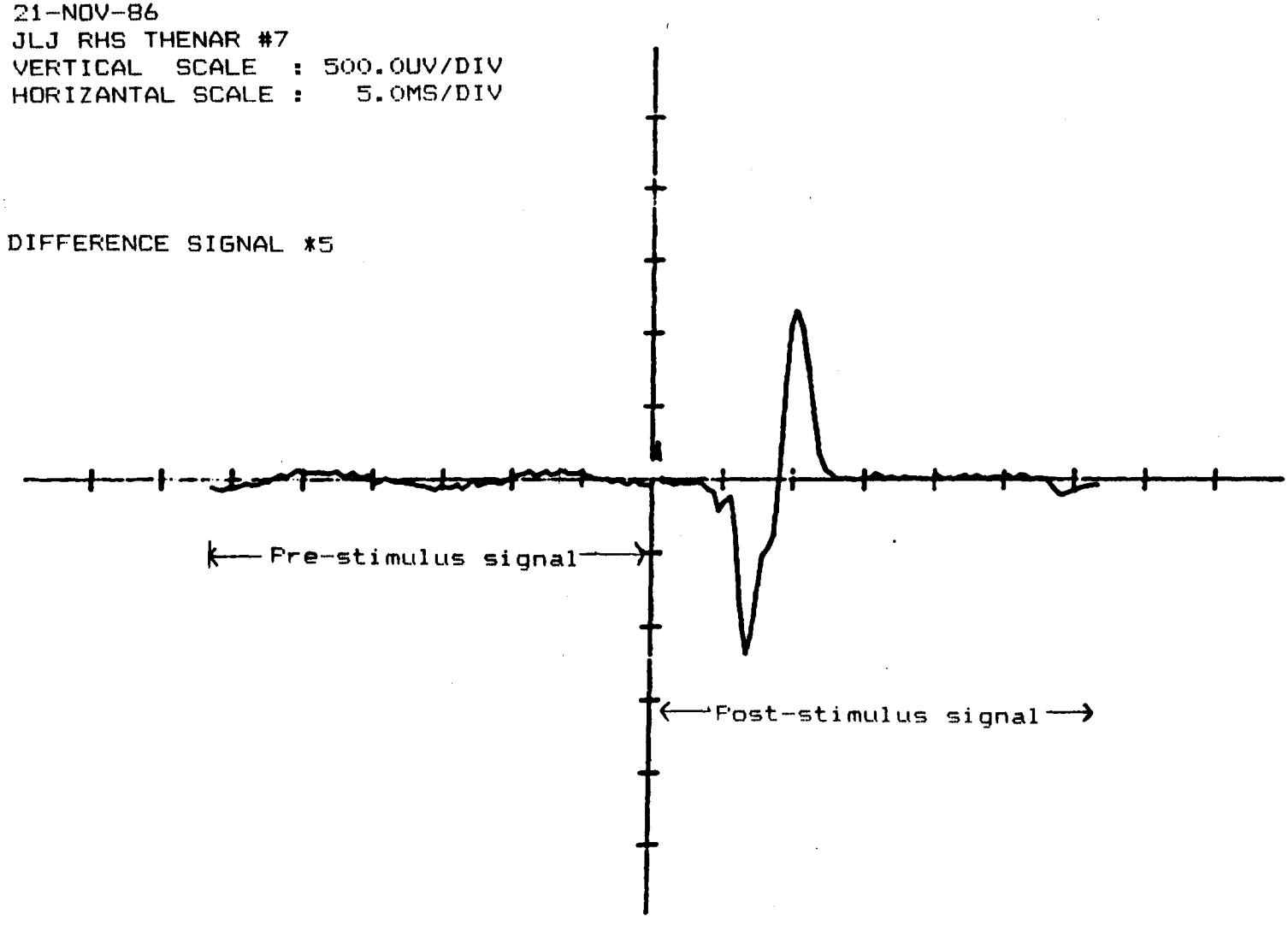


Figure 4-6 Display of Evoked Response and Difference Signal

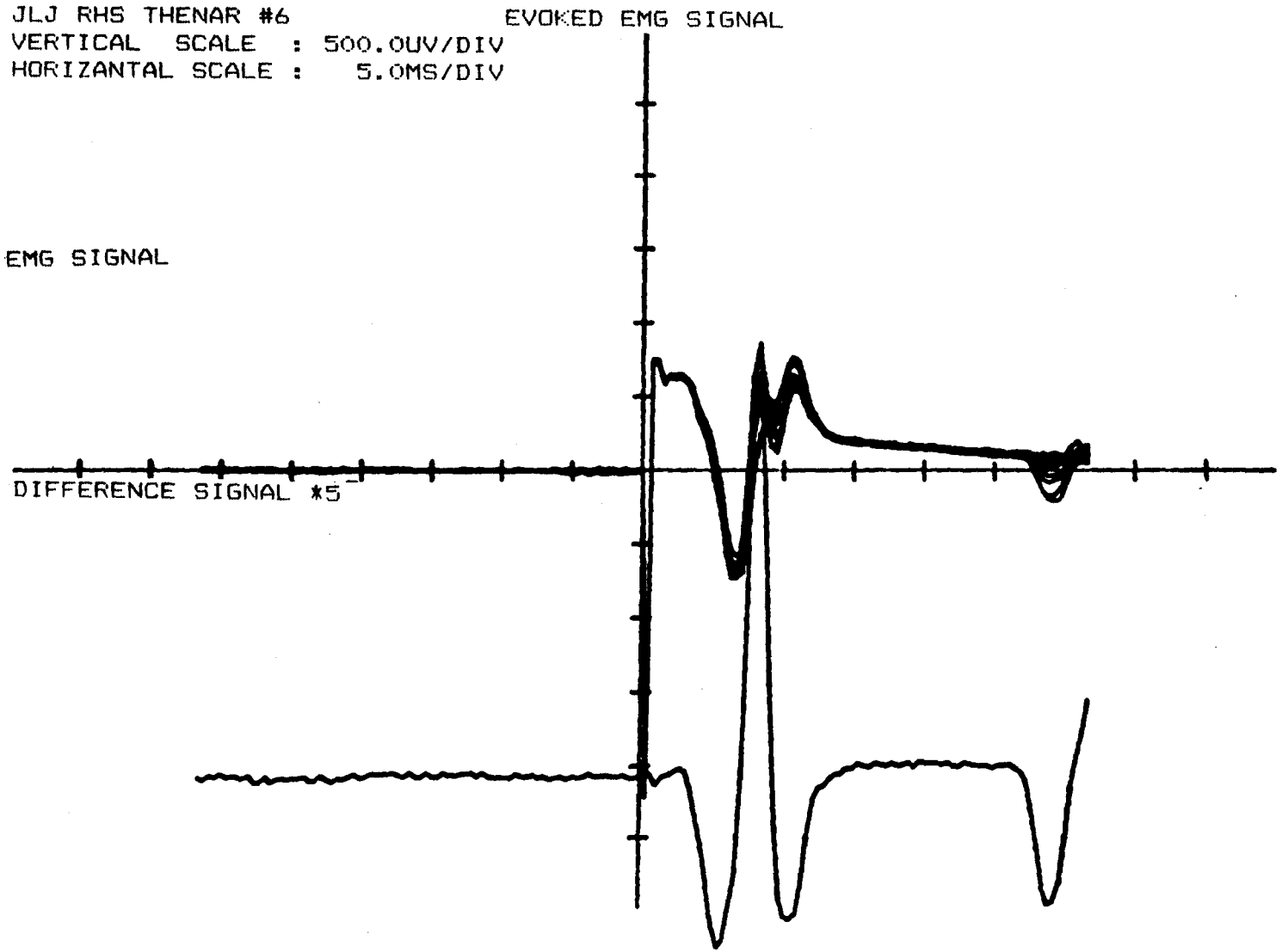
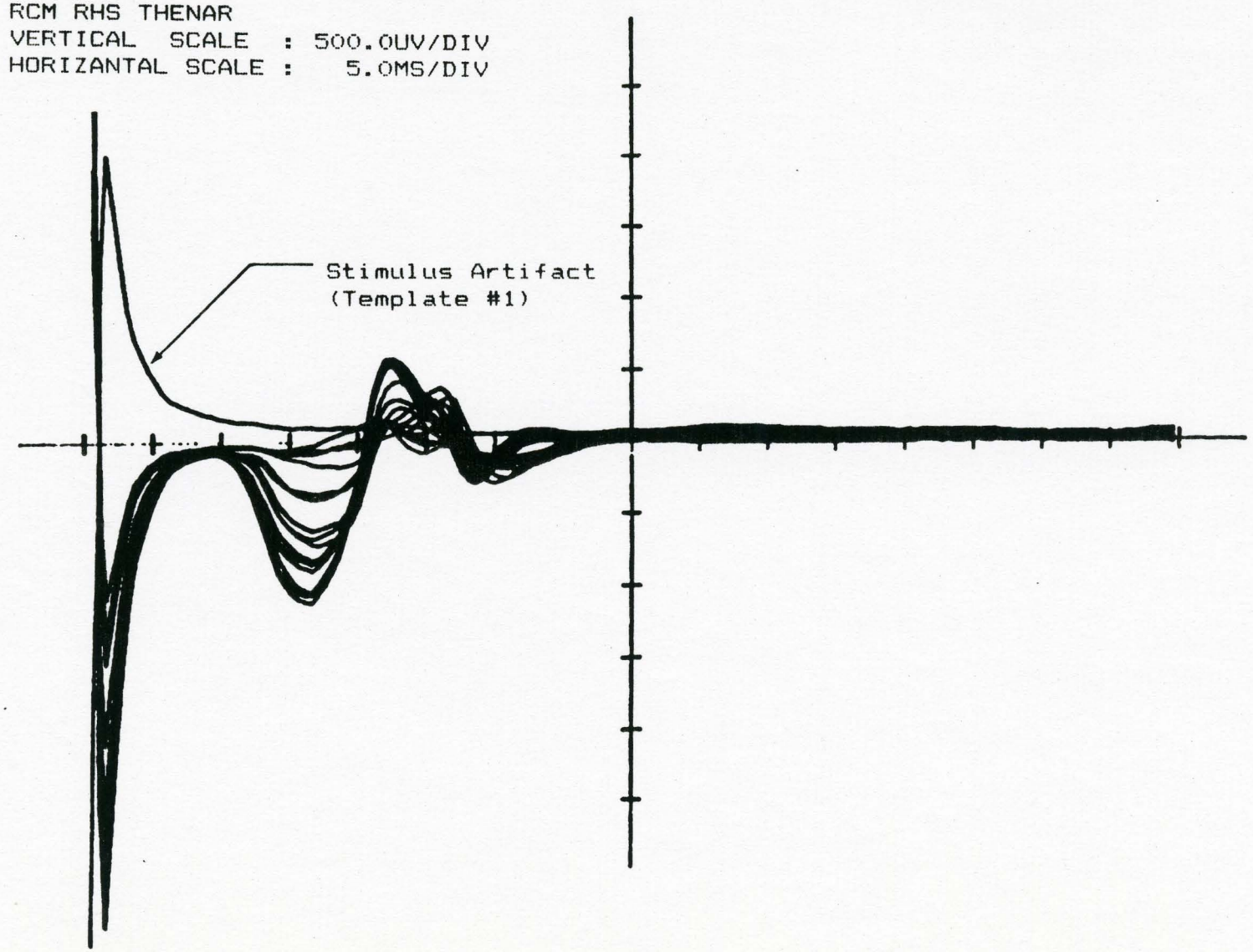


Figure 4-7 Display of Templates



Figures 4-4 to 4-7 were all acquired from the program AVMUAP. Figure 4-4 shows a display of the evoked responses. This display could be disabled to reduce the inter-stimulus time. If disabled, a signal was only sent to the graphics display screen when it did not match an existing template. In this case, the signal displayed would be the difference between that evoked response and the nearest template as shown in Figure 4-5. If the display was not disabled, the difference signal would be displayed on the same screen as the evoked response as shown in Figure 4-6. In both cases, the difference signal's amplitude is expanded by a factor of five, as indicated on the display legend (Figures 4-5 and 4-6) by the note "DIFFERENCE SIGNAL *5". When the program AVMUAP.FOR was completed, all templates were displayed on the graphics screen as shown in Figure 4-7.

Figure 4-8 shows the display of all responses evoked by the program MAXEP.FOR. This figure shows three smaller amplitude responses evoked as the stimulus was increased. The wide band of overlapping signals represents twenty five samples of the MEP. Figure 4-9 shows the ensemble average of these twenty five samples.

Figure 4-8 Display of Responses Evoked During MAXEP.FOR

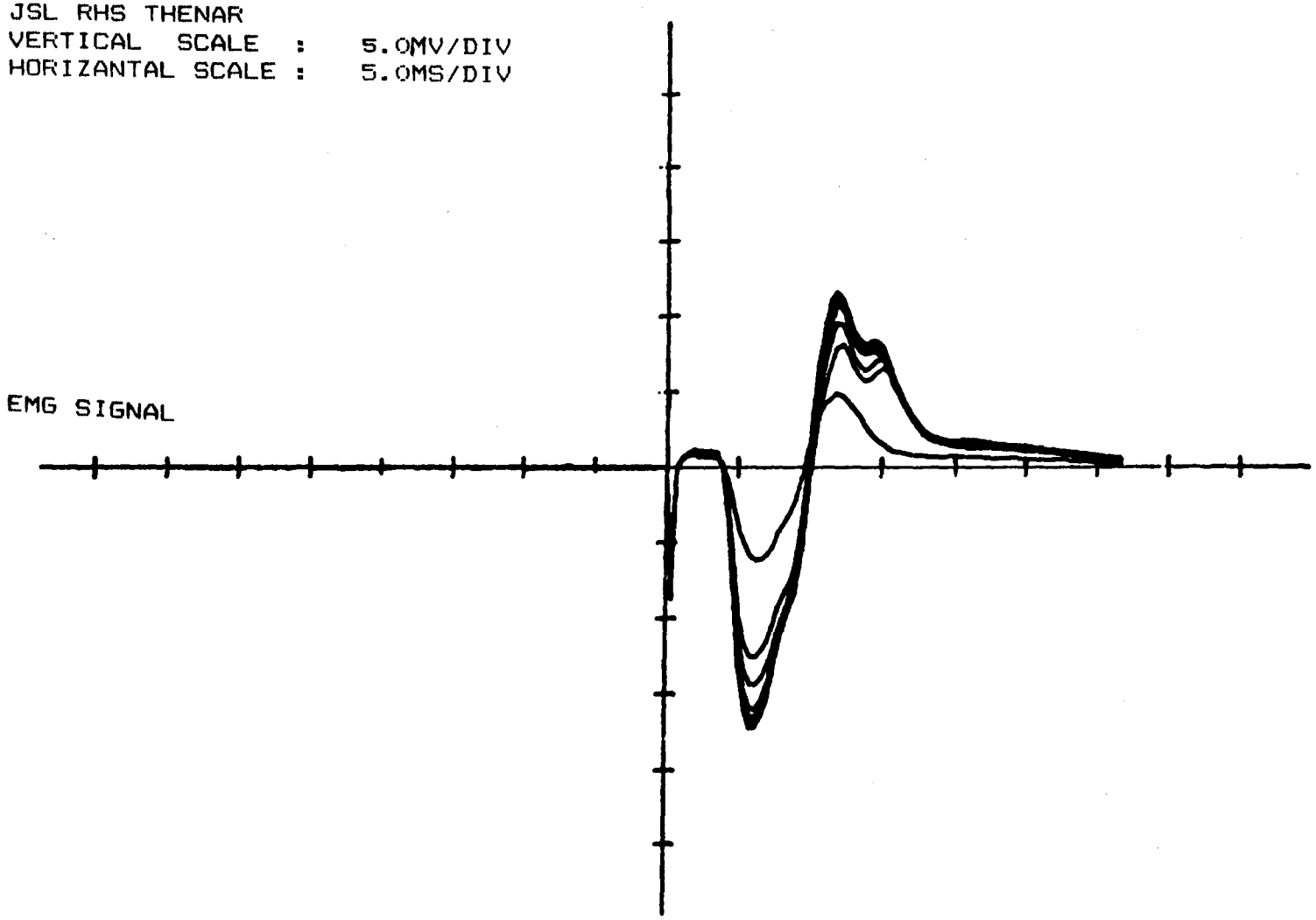


Figure 4-9 Ensemble Average of MEP Responses

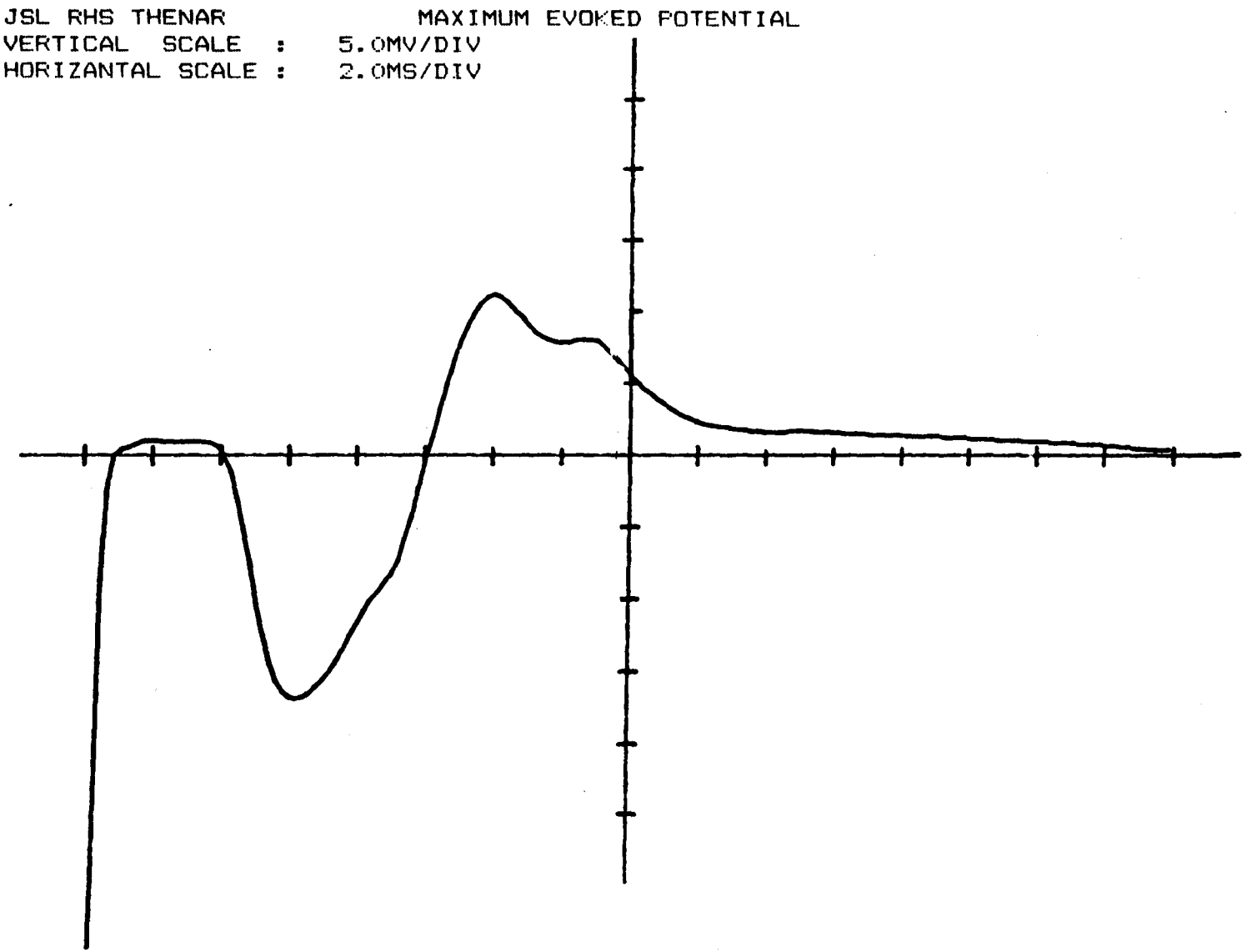


Figure 4-10 Signals Recovered by Successive Subtraction

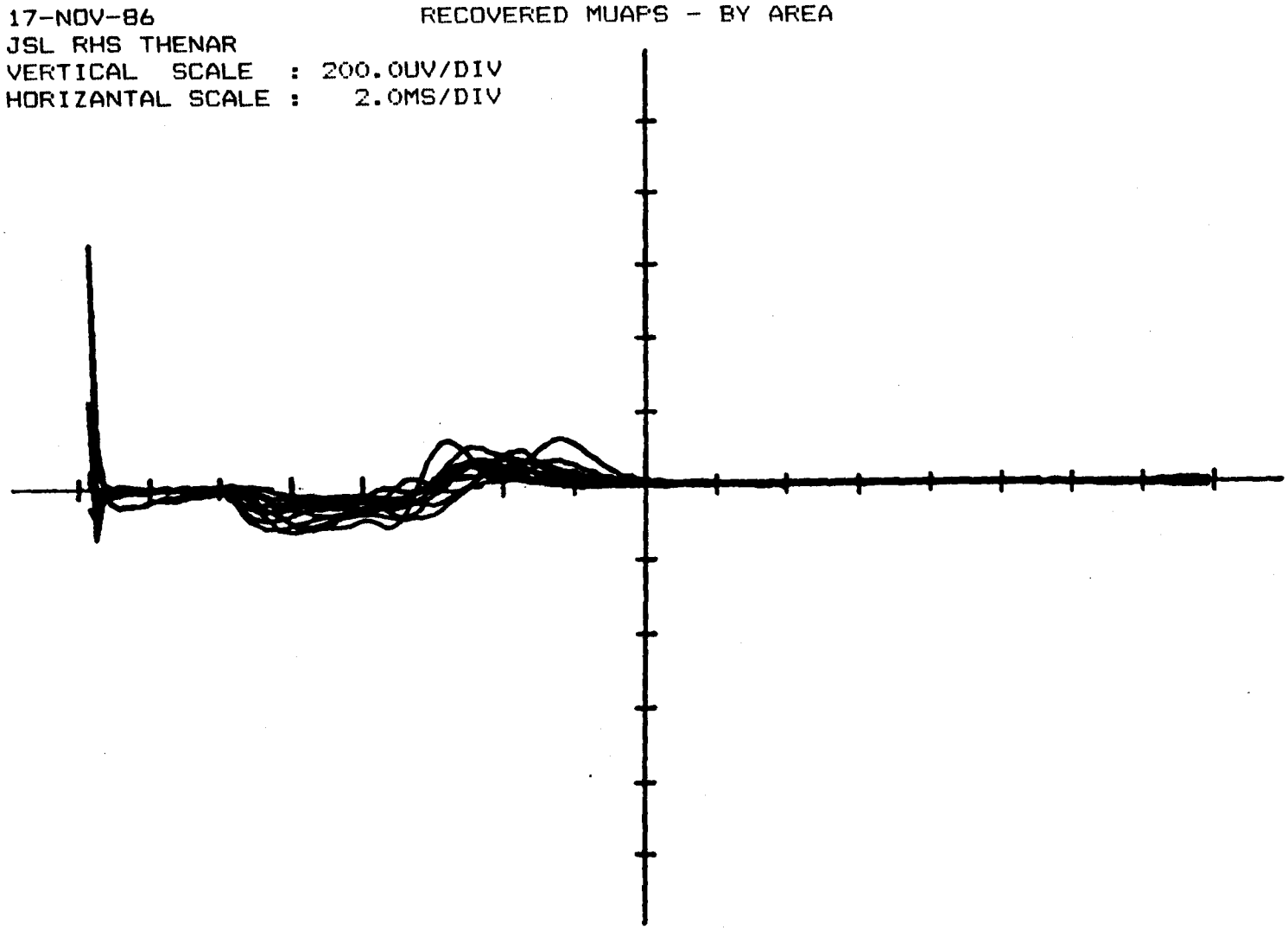


Figure 4-11 MEP Response vs MEP Estimated Using Regression

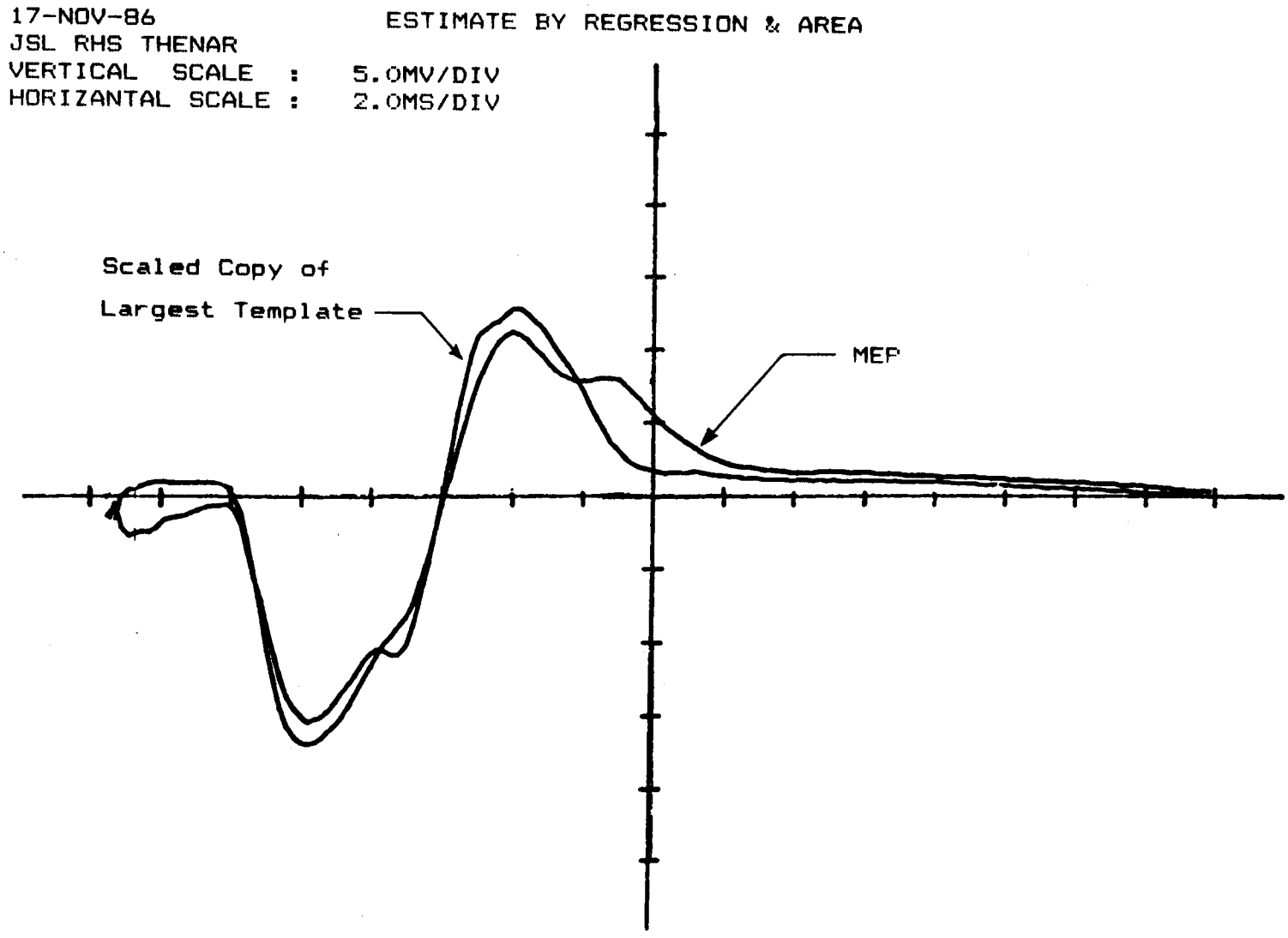


Figure 4-12 MEP Response vs MEP Estimated Using McComas Method

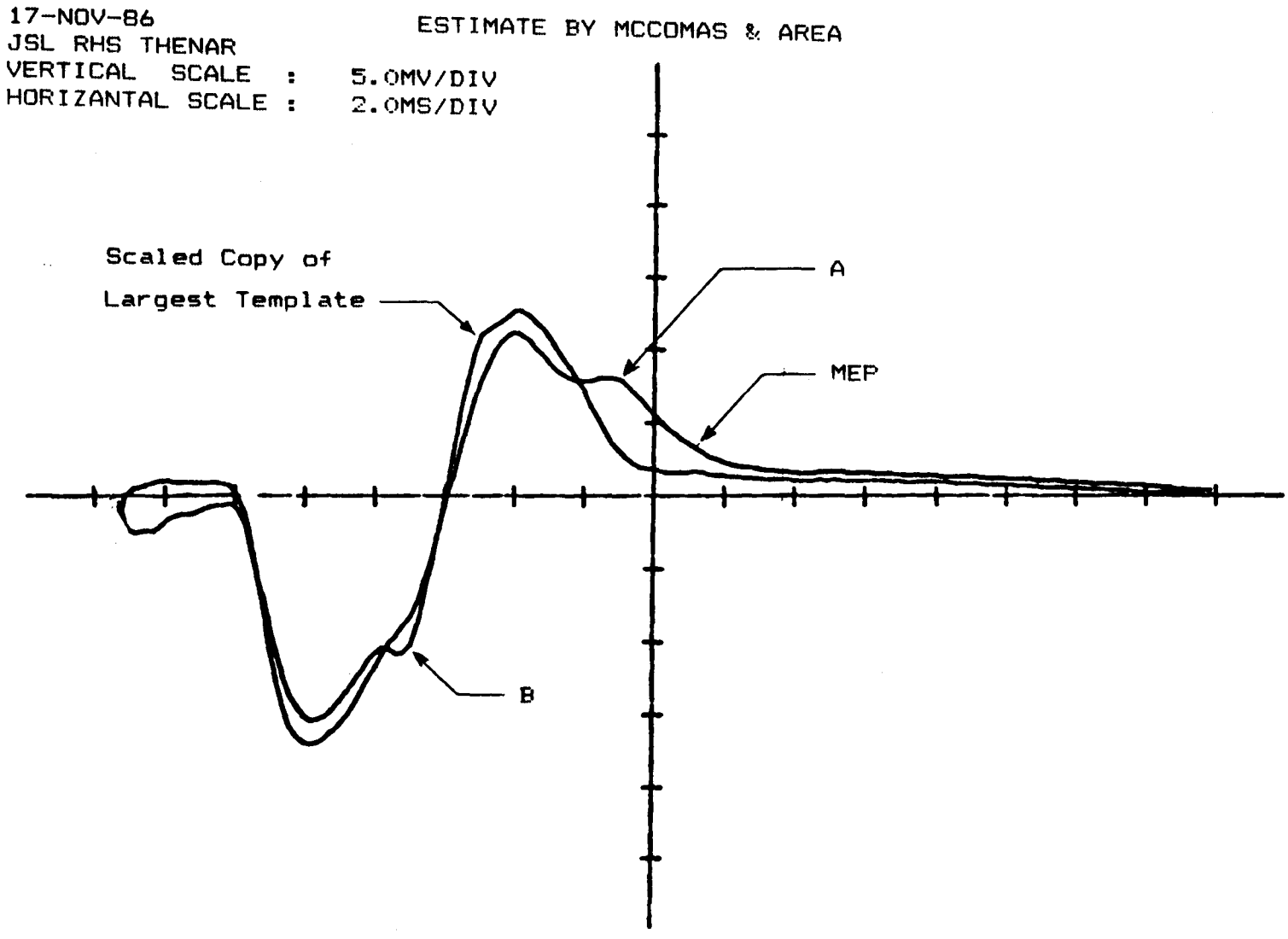
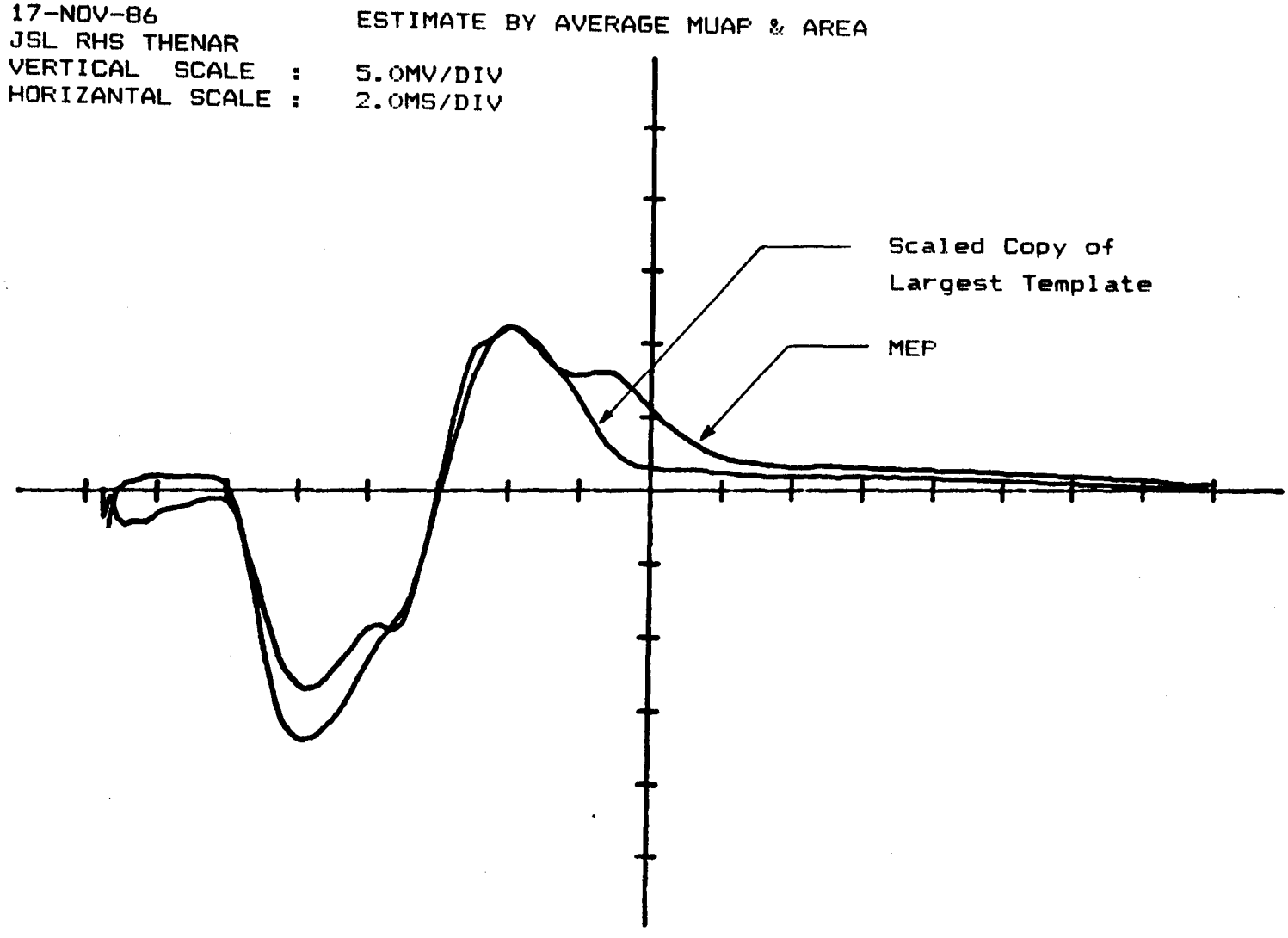


Figure 4-13 MEP Response vs MEP Estimated Using Average Method



Figures 4-10 through 4-13 were produced by PROCES.FOR as it calculated the estimates based on area. Equivalent figures, not shown here, were produced by this program as it calculated the estimates based on peak. Figure 4-10 shows the MUAPs recovered by successive subtraction of the templates ordered by area. For this data, identical results were obtained when the templates were ordered by peak. Figure 4-11 shows two signals, the MEP and a copy of the largest (by area) template scaled using the motor unit estimate obtained from the regression method. Figure 4-12 is the same as Figure 4-11 except the largest template is scaled up by a factor proportional to the estimate by McComas, rather than by the estimate by regression. In Figure 4-13, the scaling factor used is proportional to the estimate obtained by the average MUAP method.

4.4. Sources and Effects of Signal Noise

As discussed in previous chapters, there are four sources of noise which can cause errors in the template matching operations of the motor unit counting system. These are 60 Hz interference, stimulus artifact, background EMG and the H-reflex.

The effects of 60 Hz interference were minimized by using amplifiers with high common mode rejection. As discussed in Chapter 4, the effects of any remaining 60 Hz interference on the identification of a change in the post stimulus signal were minimized by cancellation by synchronizing the stimulus to the line frequency. Figure 4-5 demonstrates the cancellation of 60 Hz interference evident in the pre-stimulus signal

(approximately 30 μV p-p) but which has been removed in the post stimulus signal.

Template #1 shown in Figure 4-7 established an estimate of the stimulus artifact. This template was subtracted from the other templates shown so that they represent only the evoked EMG. The result of the subtraction of the stimulus artifact is that the remaining templates lie on the zero base line. The remaining artifact in the region from 0-2 ms. demonstrates the slight error in the estimation of the stimulus artifact. Each of the 17 templates shown in Figure 4-7 represents the ensemble averages of between six and eighty signals.

Figure 4-4 shows the consistent presence of the H-wave. In these tests, the window within which the signal features were calculated extended from 4 mS to 25 mS. The H-wave would therefore not affect the template assignment process. This window also excluded any vestigial stimulus artifact remaining after the cancellation discussed above.

Errors in the template matching process due to background EMG were minimized by the rejection of signals which showed excessive pre-stimulus noise. Table 4-2 shows a typical assignment of signals. Only those signals which were assigned to templates other than Template #1 contributed to the estimate of the average MUAP. The numbers in the first four categories of the table are small, indicating that the subject was relaxed and there was little noise from other sources. Alternately, these low numbers could indicate that the thresholds were excessively high. Signals assigned to the "No Match Found" category represent the responses which were not reproducible, either due to noise or to alternation. Signals assigned to Template #1 were used to estimate the stimulus

artifact. The number of signals falling into this category reflect the accuracy of the estimate of the motor threshold.

Table 4-2: Typical Signal Assignments (Subject I.B.)

Baseline Drift	0	0%
A/D Saturation	0	0%
Excessive Noise(1)	12	2%
Excessive Noise(2)	0	0%
No Match Found	139	22%
Assigned to Template #1	47	7%
Assigned to Other Templates	435	69%
Total Signals Sampled	633	100%

4.5. Errors in the Template Creation Process

There are two types of errors to be dealt with, Type I and Type II. A Type I error occurs when a new motor unit is evoked but a template is not created. A Type II error occurs when a template is falsely created.

Figure 4–14 **Clustering of Responses by Area**

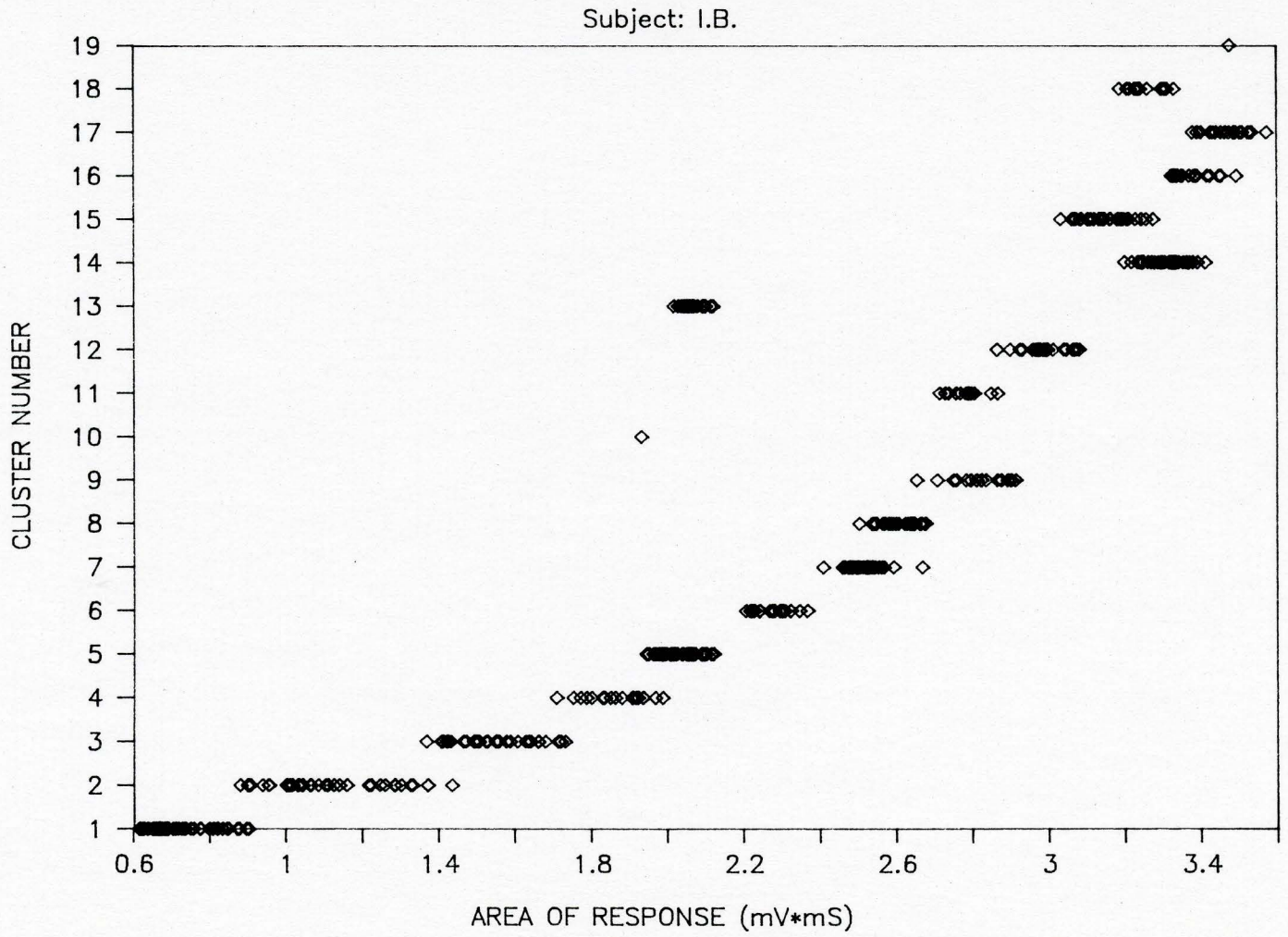
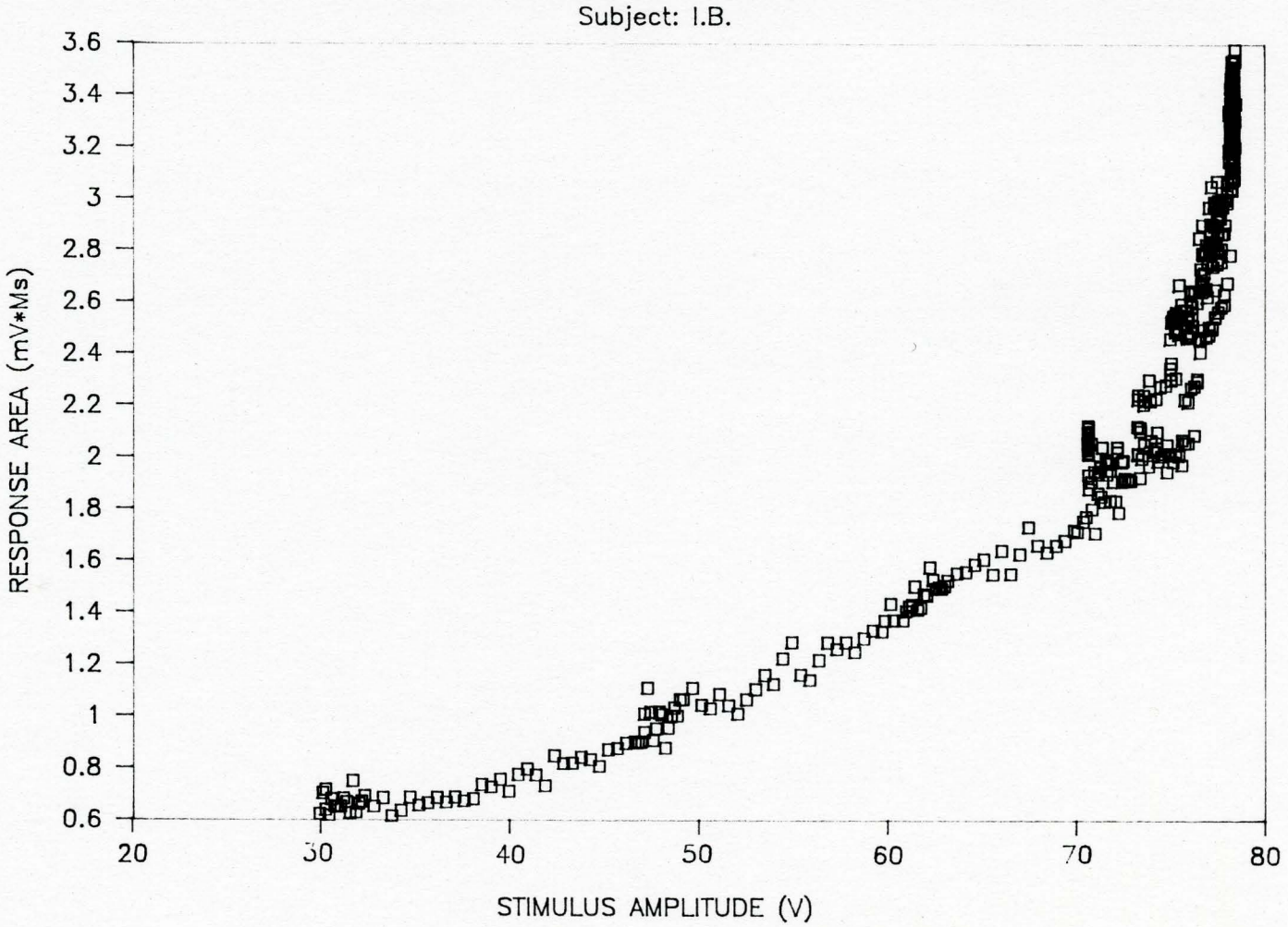


Figure 4–15 Area of Response vs Stimulus Amplitude



4.5.1. Type I Errors

Type I errors occur when the size of the new motor unit is of the same magnitude as the noise. Figure 4-14 indicates the degree of the intra-cluster scatter of the samples. Rather than a function having discontinuities for each cluster, Figure 4-15 shows a continuous, direct relationship between stimulus amplitude and response area. The intra-cluster r^2 values (Table 4-3) for the data are statistical measures of a direct relationship which may be due to stimulus artifact or may indicate the existence of an unidentified increment in the response. The importance of using a range of stimulus values to acquire samples all subsequently assigned to a given template was that it permitted the calculation of a meaningful r^2 which a single stimulus value would not accomplish.

Table 4-3: Correlations of Response Area vs. Stimulus Amplitude

Template	Samples	r^2	Significance
1	47	0.776	Significant (p<.01)
2	41	0.865	Significant (p<.01)
3	32	0.855	Significant (p<.01)
4	22	0.435	Significant (p<.01)
5	46	0.002	N.S.
6	21	0.165	N.S.
7	30	0.159	Significant (p<.05)
8	26	0.013	N.S.
9	21	0.067	N.S.
11	14	0.113	N.S.
12	29	0.248	Significant (p<.01)
13	25	0.000	N.S.
14	44	0.034	N.S.
15	33	0.002	N.S.
16	17	0.062	N.S.
17	19	0.331	Significant (p<.01)
18	15	0.014	N.S.
MEP	25	0.143	N.S.

4.5.2. Type II Errors

When a template is falsely created, it is likely abandoned due to insufficient recruitment of members, as occurred, for example, in Template #10 for subject I.B.. This template represented a single evoked response which occurred twice in succession, but was then not reproducible and was therefore discarded. However, it is also possible to encounter a situation where two templates are formed which are not significantly different. Such may have been the case for Templates #13 and #5 for subject I.B..

4.6. Motor Unit AP Templates

The motor units recruited to estimate the average motor unit action potential (MUAP) must be representative of the MU's in the muscle in order to obtain an accurate estimate. Figure 4-16 shows the templates evoked from subject JSL. With these templates rank ordered by area, the differences between adjacent templates are assumed to be MUAPs. Subtraction of adjacent templates yielded the signals shown in Figure 4-10. A bias towards either large or small MU's evoked by stimulus amplitudes close to the motor threshold may exist. This can be investigated by determining the relationship between the order by which the MU's were evoked and the stimulus amplitude required to evoke them. Table 4-4 shows a summary of the correlation between the area of the recovered templates and their ranking order. Data exhibiting a strong correlation suggest that the corresponding estimates may be in error due to biased sampling of the MUs.

Figure 4-16 Templates from Subject JSL

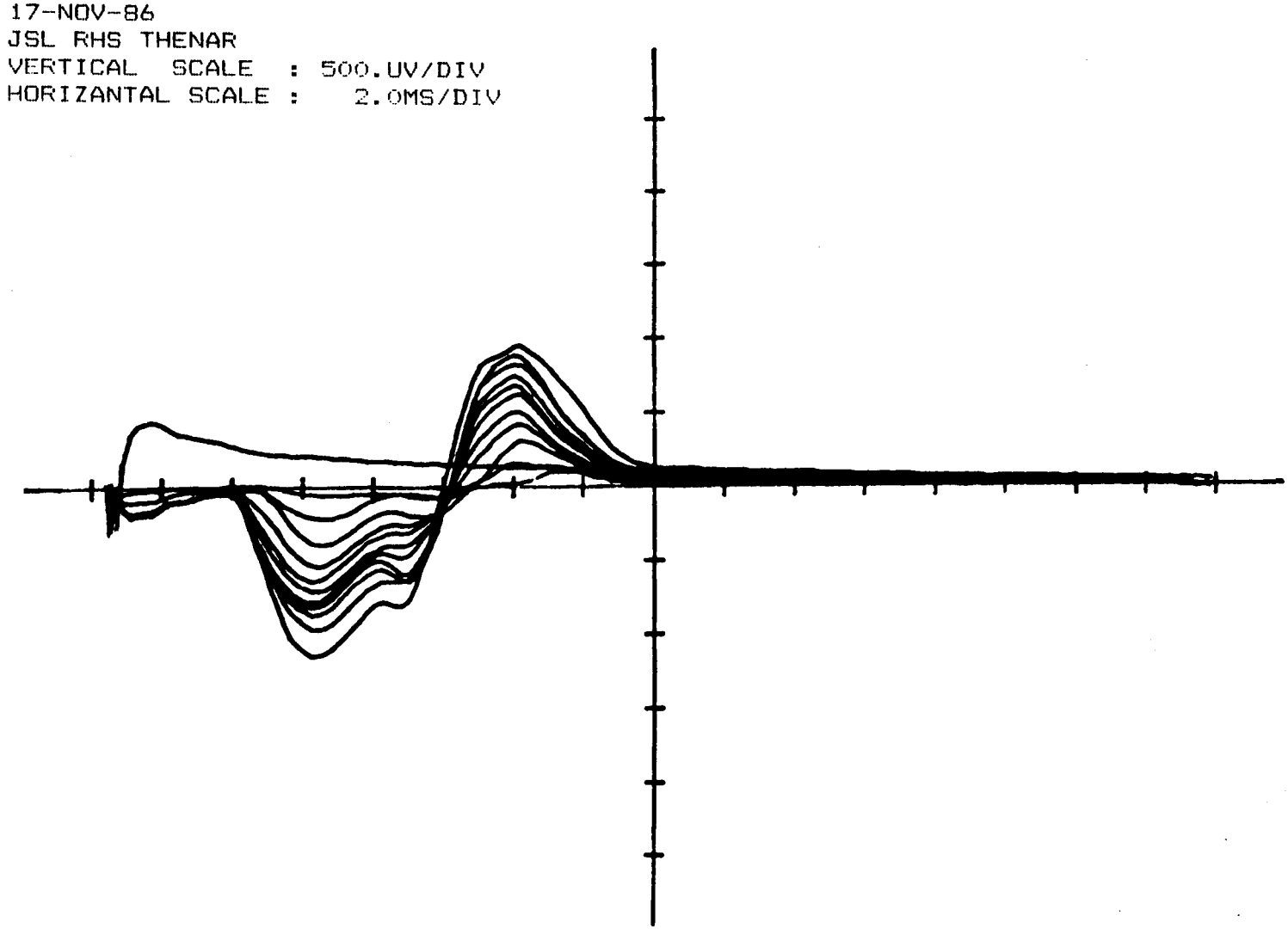


Table 4-4: Correlation Between Area of MUAP and Ranking Order

Test	MUAPs	r^2	Significance
JLJ #3	16	0.000	N.S.
JLJ #4	11	0.370	N.S.
JLJ #5	15	0.308	Sig. ($p<.05$)
JLJ #6	7	0.109	N.S.
JLJ #7	16	0.144	N.S.
JLJ #8	9	0.143	N.S.
LMO	16	0.042	N.S.
JSL	11	0.004	N.S.
ION	16	0.557	Sig. ($p<.05$)
NPP	13	0.572	Sig. ($p<.05$)
RCM	16	0.276	Sig. ($p<.05$)
BJM	10	0.047	N.S.
DBM	5	0.939	Sig. ($p<.05$)
JGJ	17	0.018	N.S.

Another indicator for the presence of bias in the MUAP sampling is found in Figure 4-12 which shows two curves, the MEP and a scaled copy of the largest of the templates evoked. The scaling factor used was the estimate of the number of MU's in the muscle divided by the number of MU's assumed to have formed the template. The similarity in shape and latency between these two curves is an indication of the representativeness of the MU's sampled during the estimation process - those MUs which form the largest template recruited. The secondary peaks "A" and "B" shown in Figure 4-12 indicate discrepancies which may be because of a variation in the shapes of the MUs recruited, but not apparent in the MEP because of the averaging effects of the summation. Alternatively, the discrepancy may be caused by a bias in the recruiting characteristics of the lower amplitude stimuli.

Figure 4-17 Signals Evoked at a Constant Stimulus Amplitude

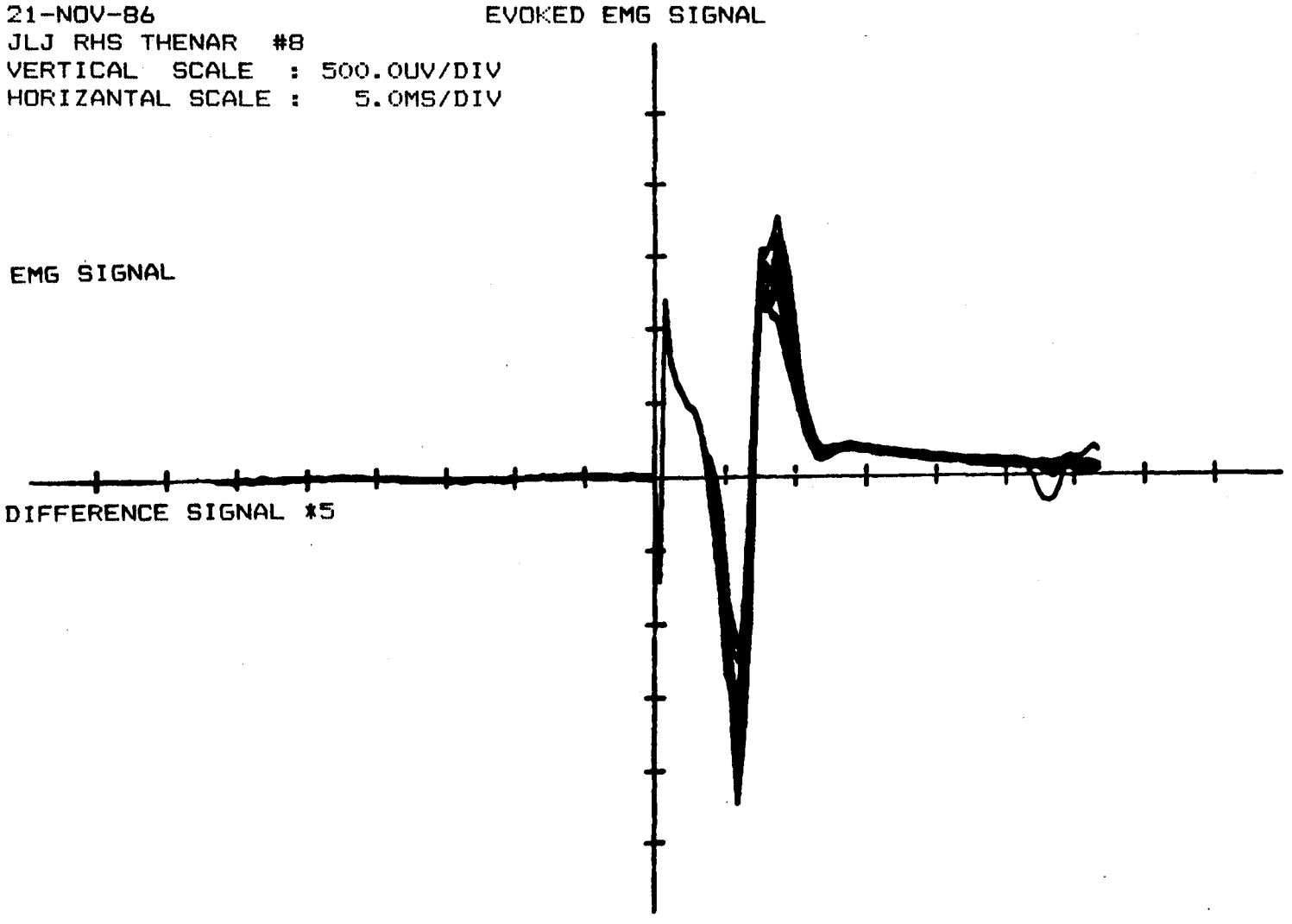
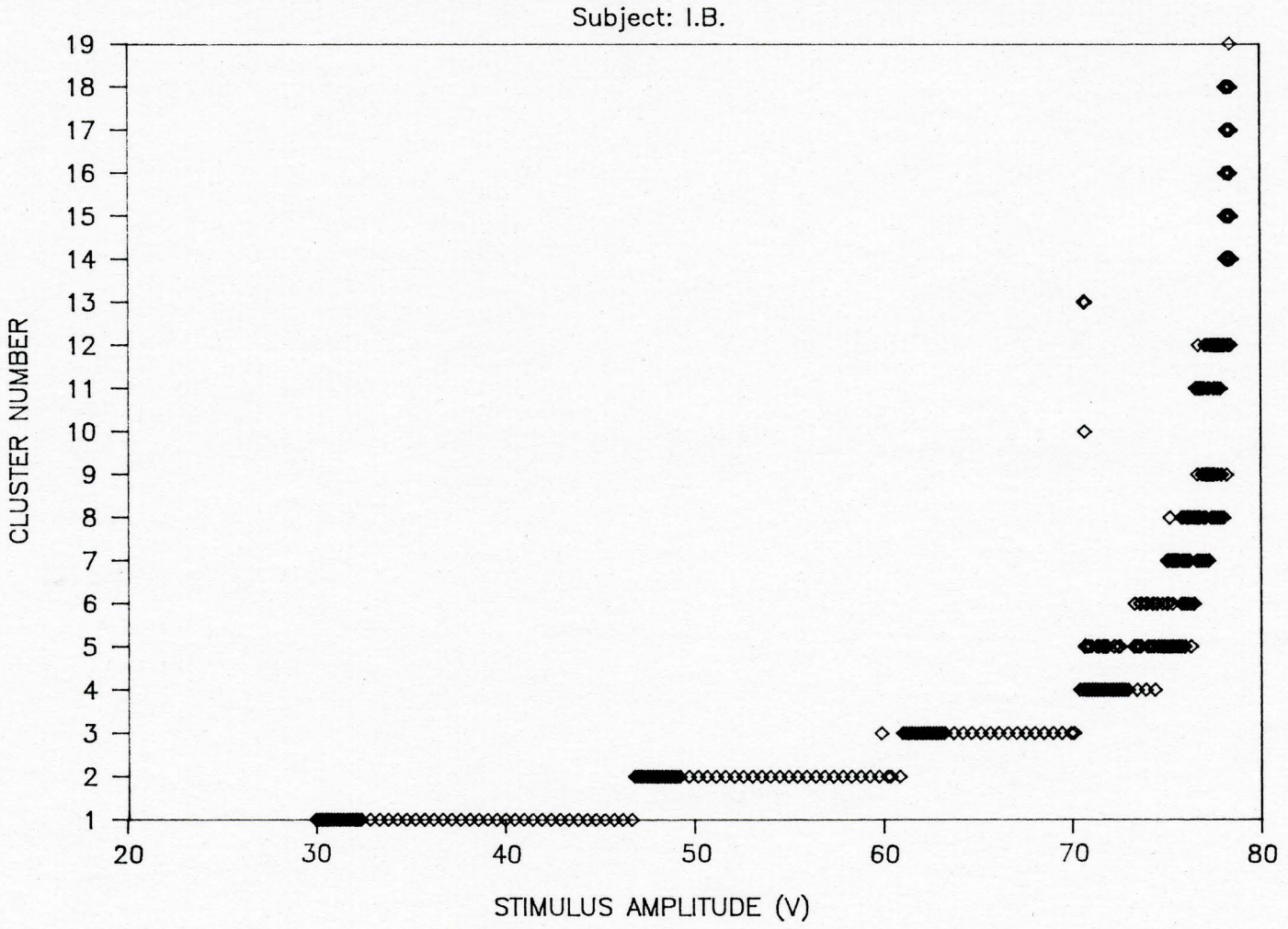


Figure 4–18 Cluster Assignment / Stimulus Amplitude



4.7. Alternation

Alternation was made evident by the appearance of a small family of responses consistently evoked at a constant stimulus. Figure 4-17 represents approximately 150 signals all evoked with a constant stimulus amplitude. The responses all appeared to fall into one of approximately 5 to 8 shapes. Generally, during alternation at least two responses which do not differ significantly could be evoked consecutively. This would cause the formation of a new template. The remaining responses apparent during alternation may not have occurred twice in succession, thus no template would be formed to capture them. In four of the thirteen tests, the alternation was such that two consecutive responses which were not significantly different would not be evoked within a reasonably long time - approximately two or three minutes. Hence, no new template was formed and the program was stopped for this reason. In these test runs, the "No Match Found" (Table 4-2) incidence approached 100%.

The effect of alternation was more apparent at increased stimulus amplitudes. Figure 4-18 shows an increasing number of clusters having overlapping stimulus amplitude ranges at the larger stimulus amplitude values. The overlapping of the clusters as projected onto the stimulus amplitude axis is an indication of alternation. This may be because of the instability of the thresholds of the individual MU's involved ("true" alternation) or because the MU's involved all have motor thresholds which are constant but nearly identical. In the latter case, a similar vari-

ation in the evoked response would result from any small changes in the apparent stimulation level due to electrode motion etc..

4.8. The Assumption of Linear Summation of Features

Two topics will be discussed in this section. The first concerns the magnitude of the error caused by nonlinear summation of the features used to describe the responses. The second is factors which contribute to nonlinear summation.

4.8.1. Indications of Error Due to Nonlinear Summation of Features

Figure 4-19 shows the relationship between the number of MU's contributing to the summated response, and the area of the response. Perfect linearity would be evident by a straight line relationship between the number of MU's and the area of response. A statistical measure of the magnitude of the deviation from perfect linearity is the coefficient of determination (r^2). In this example, the r^2 value was 0.89. This can be interpreted to mean that 89% of the variation in the area of response is due to the linear regression of the area of response on the number of MU's.

Figure 4–19 **Area of Response Vs Number of MUs**

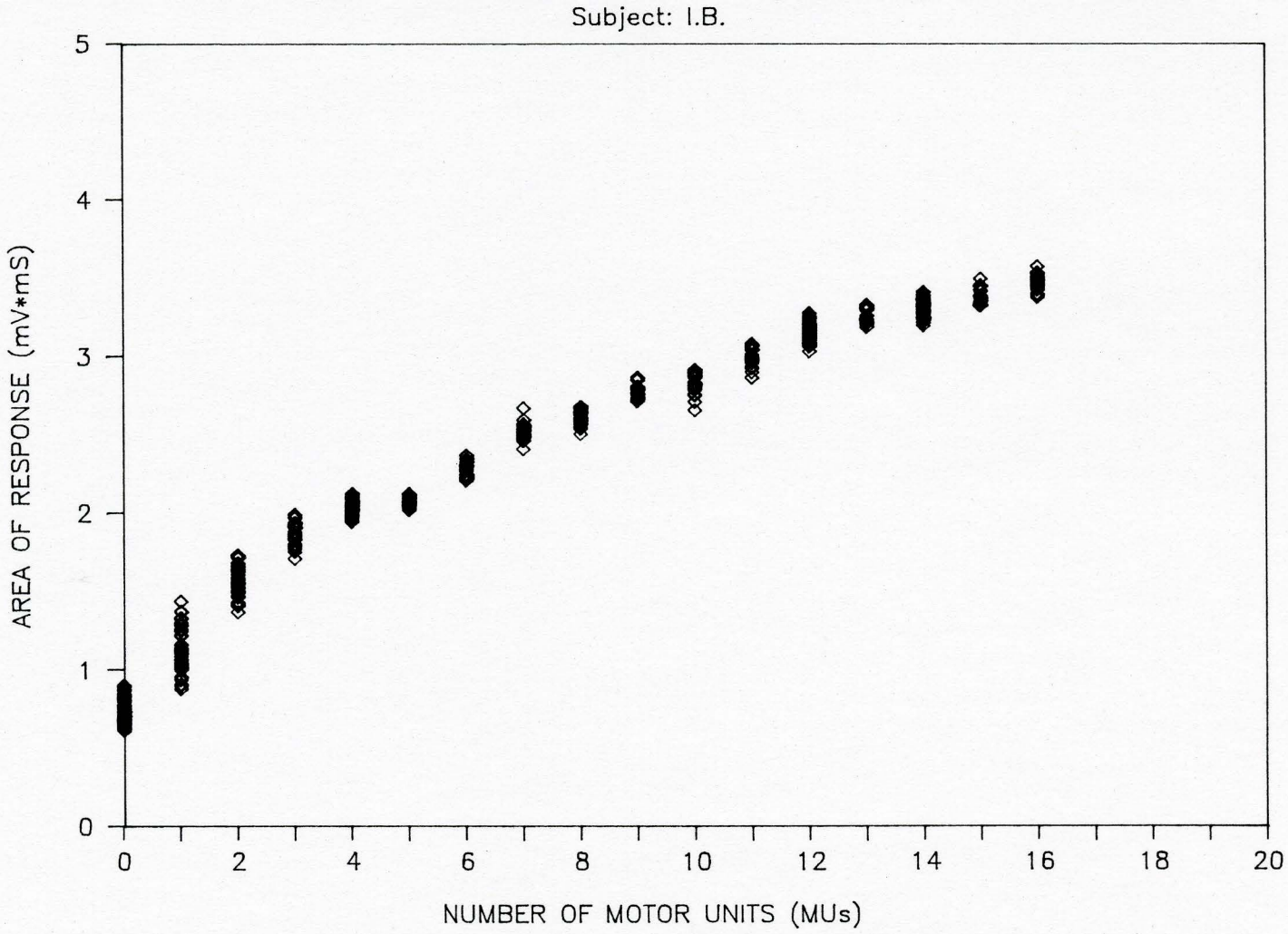


Figure 4-19 was generated by ranking the templates by area, and then assuming that each increment in area was due to the excitation of an additional MU. The negative second derivative of the curve shown in this figure can be accounted for by errors in one or both of two assumptions. First, alternation, rather than the number of MU's, may be increasing with increasing stimulus resulting in an over-estimation of the number of MU's. Alternatively, the deviation from a linear relation could be due to failure in the assumption of superposition of the MUAPs. The r^2 values for the other test runs are given in Table 4-6.

4.8.2. Factors Contributing to Nonlinear Summation of Features

As discussed in Chapter 3, variations in either the latency or the shape of the MUAPs can result in nonlinear summation of the MUAP features. The MUAPs recovered from the templates shown in Figure 4-16 are shown in Figure 4-10. They are all bi-phasic and have approximately the same shape and latencies. For Subject JSL, it is expected that the effects of nonlinear summation of features on the MU counting method would be minimal. Similar Figures, 4-20 and 4-21, for Subject BJM show recovered MUAPs which are bi-phasic but vary in latency. This variation in latency is expected to contribute to nonlinear summation. The Figures 4-7 and 4-22 for subject RCM show MUAPs which are multi-phasic. This will also contribute to nonlinear summation.

Figure 4-20 Template from Subject BJM

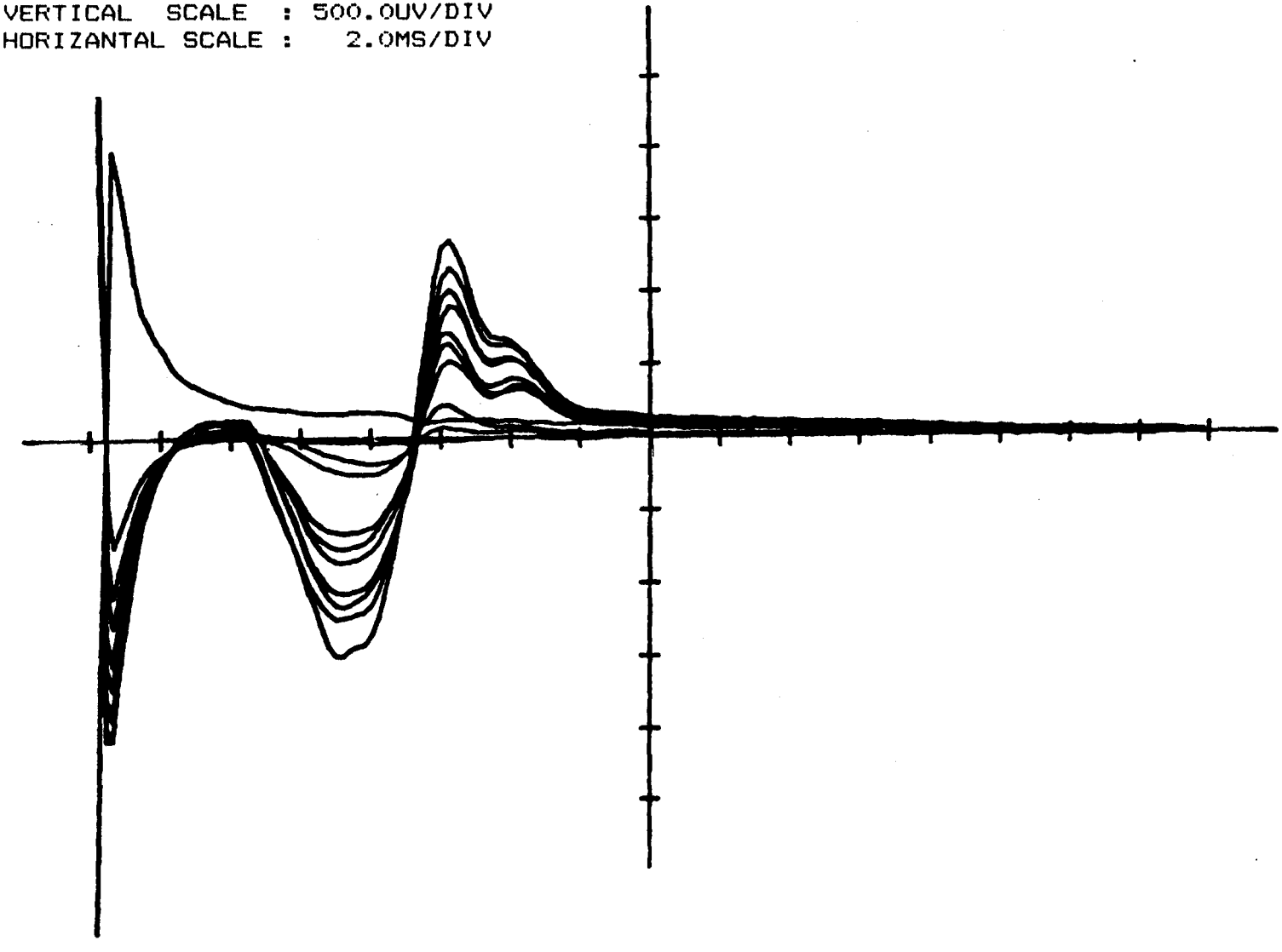


Figure 4-21 MUAPs Recovered for Subject BJM

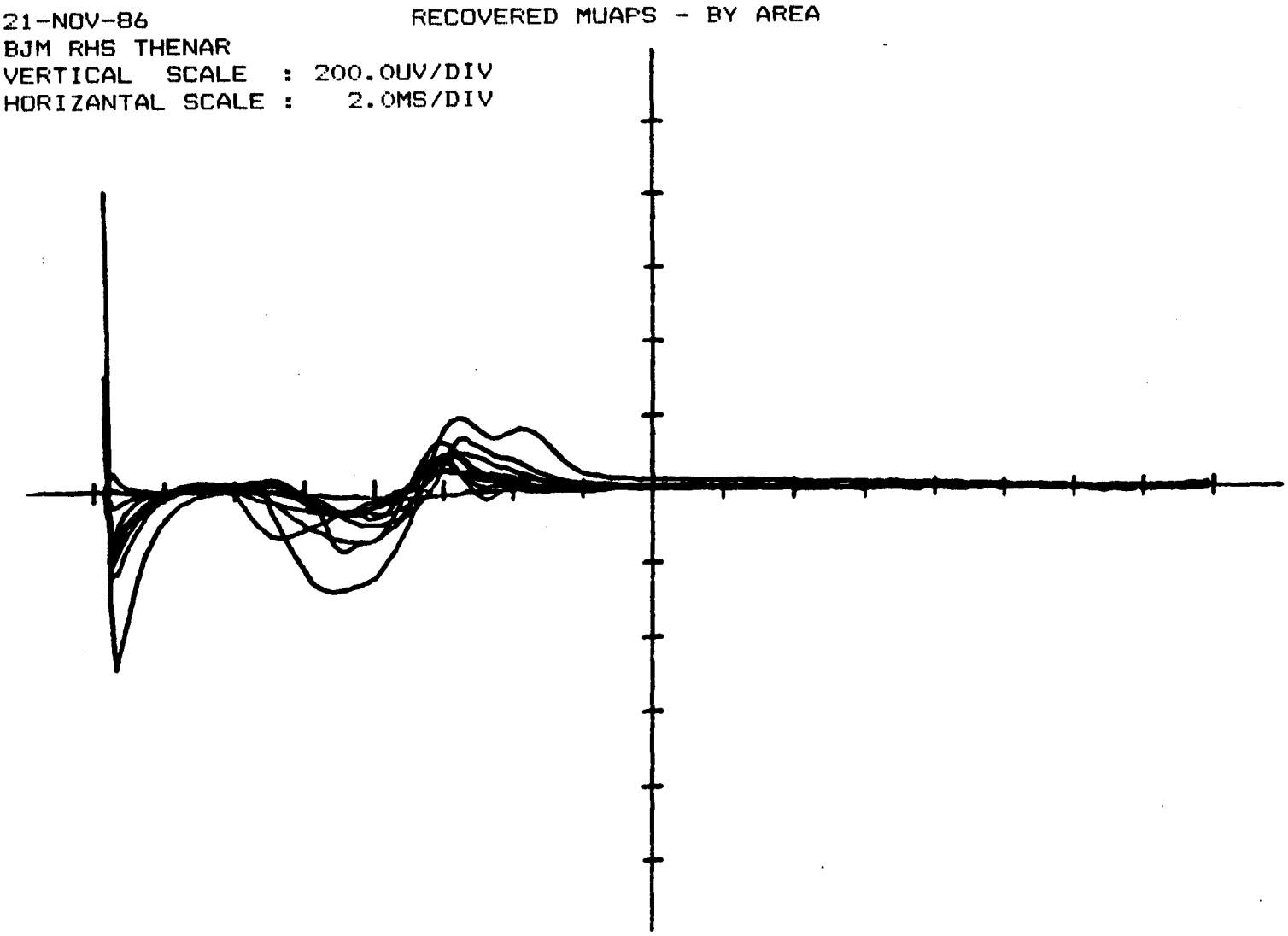
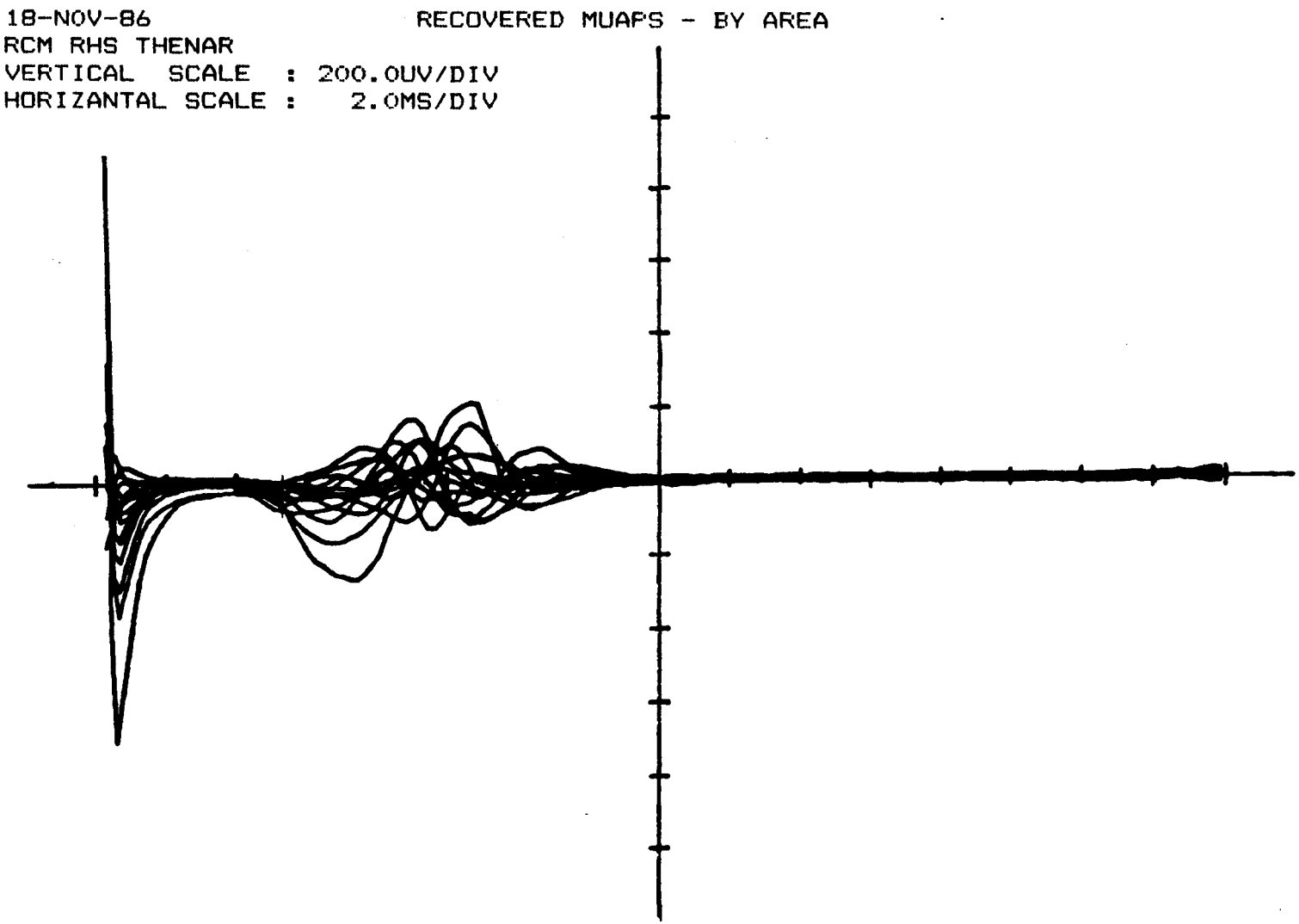


Figure 4-22 MUAPs Recovered for Subject RCM



Data for the three subjects discussed are given in the following table:

Table 4-5: Indicators of Non-linearity of Feature Summation

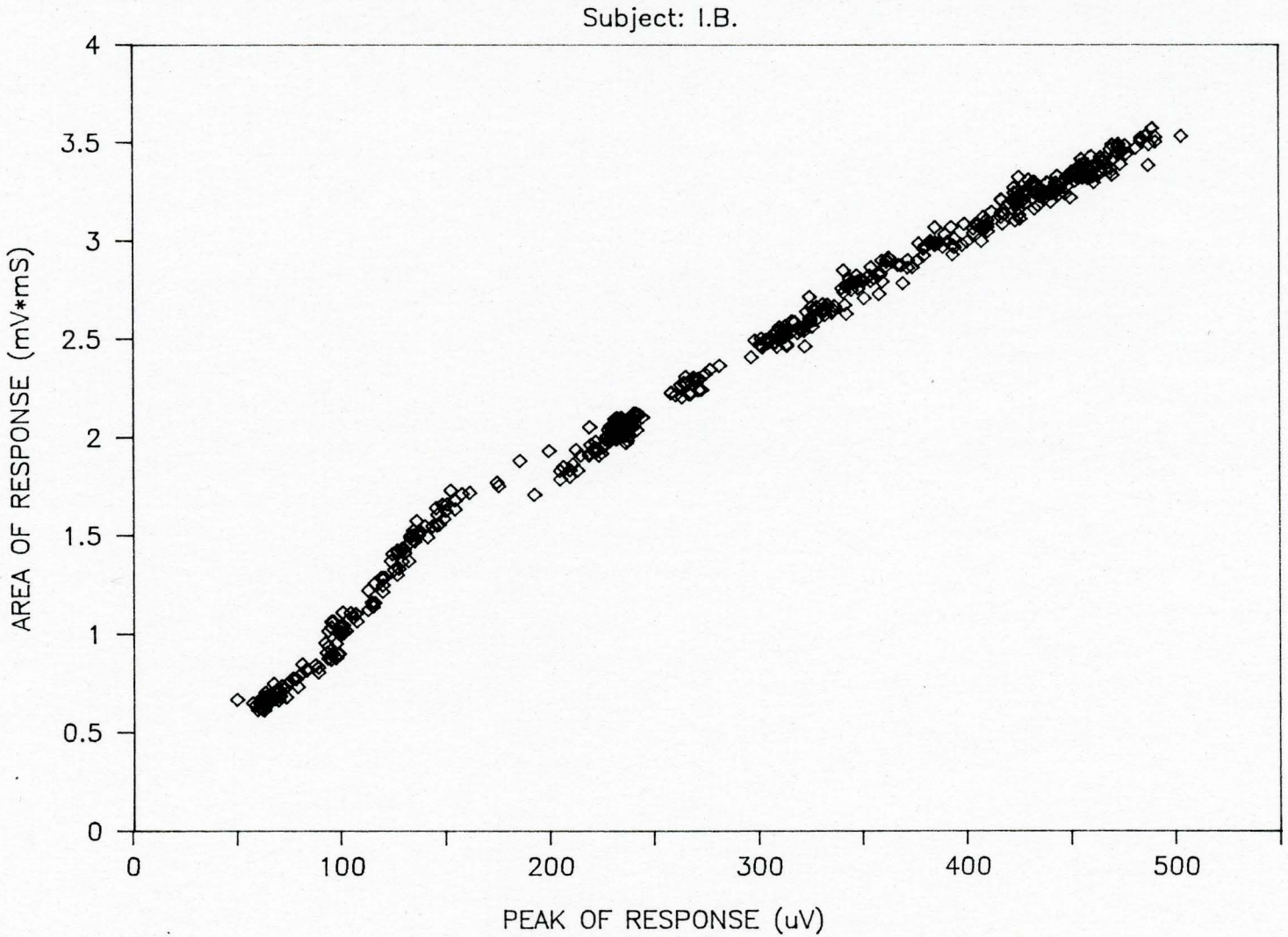
Subject	r^2	MU_M	MU_A	MU_M/MU_A
JSL	0.983	0.327	0.372	0.879
BJM	0.967	0.371	0.465	0.798
RCM	0.942	0.137	0.411	0.333

The values for MU_M are obtained by dividing the area of the largest identified template by the number of MU's assumed to have formed that template. The MU_A values were obtained by taking the average of the areas of the MUAPs recovered by decomposition of the templates. The ratio of these two values, MU_A/MU_M, may be another useful measure of linearity.

4.9. The Use of Area and Peak Amplitude

Two features, area and peak amplitude, are used a number of ways in this method. These features are used in the discrimination between responses as well as in ranking the templates and as a measure of the magnitude of the responses in the estimation calculation given by McComas. Estimates of the number of MUs using each of the features are provided by the program (Table 4-6) which will permit a comparison of the two features during clinical studies.

Figure 4–23 **Area Vs Peak of Responses**



4.9.1. Correlation Between Area and Peak

Figure 4-23 shows that area and peak were highly correlated (r^2=.99). The single large discontinuity (at 1.8 mV*mS) was caused by a change in the shape of the evoked response which altered the amplitude (peak) of the composite response but did not substantially alter the (rectified) area. In this case, the peak of the response was a slightly more sensitive feature than area. Nonetheless, the high correlation between area and peak suggests that similar performance should be attained using either feature in the estimation calculation given by McComas.

4.9.2. Ranking of Templates

Two of the estimation methods, calculation by regression and calculation of an "average" MUAP, require that the templates be ranked. Responses were ranked by either area or peak. Where the templates are well behaved in terms of shapes and latencies (Figure 4-16), it would be expected that ranking by area or by peak would result in identical ranking. Where the templates are not as well behaved (Figure 4-24), a different ranking may be obtained depending on which feature (area or peak) is used. For both estimations, templates must be correctly ordered to get correct estimates.

Figure 4-24 Templates from Subject JLJ #8

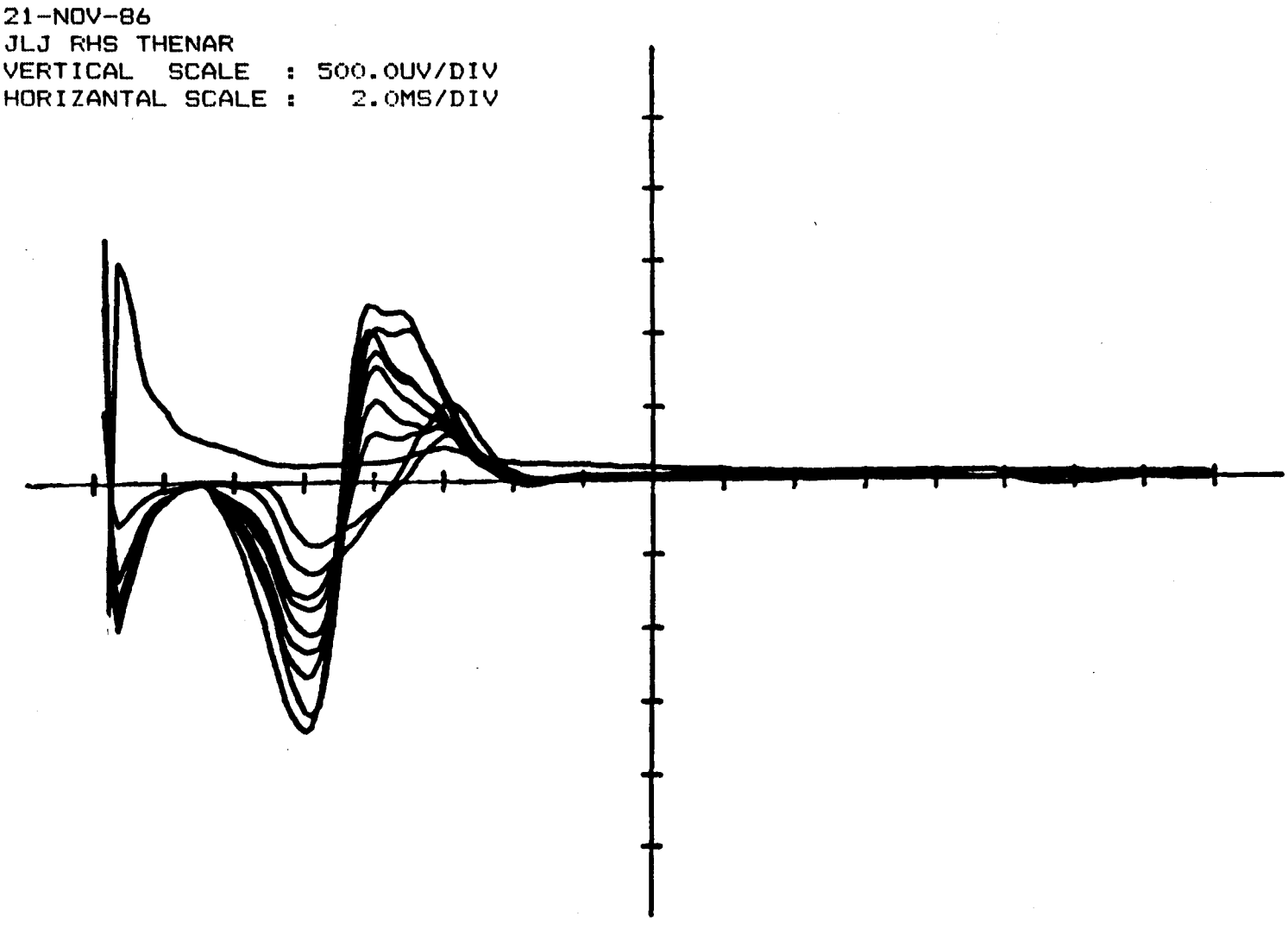


Figure 4-25 Recovered MUAPs - Ranking by Area

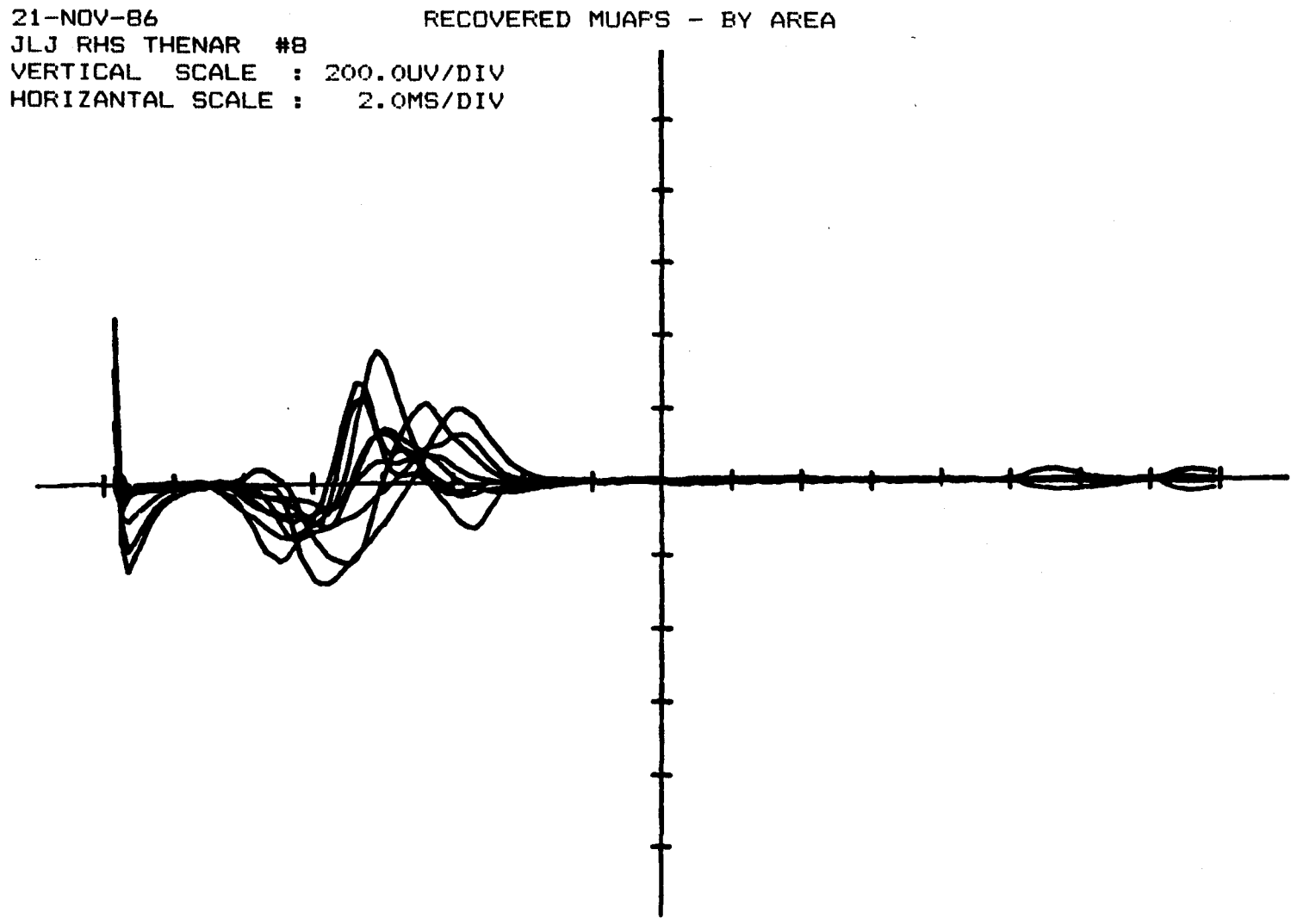
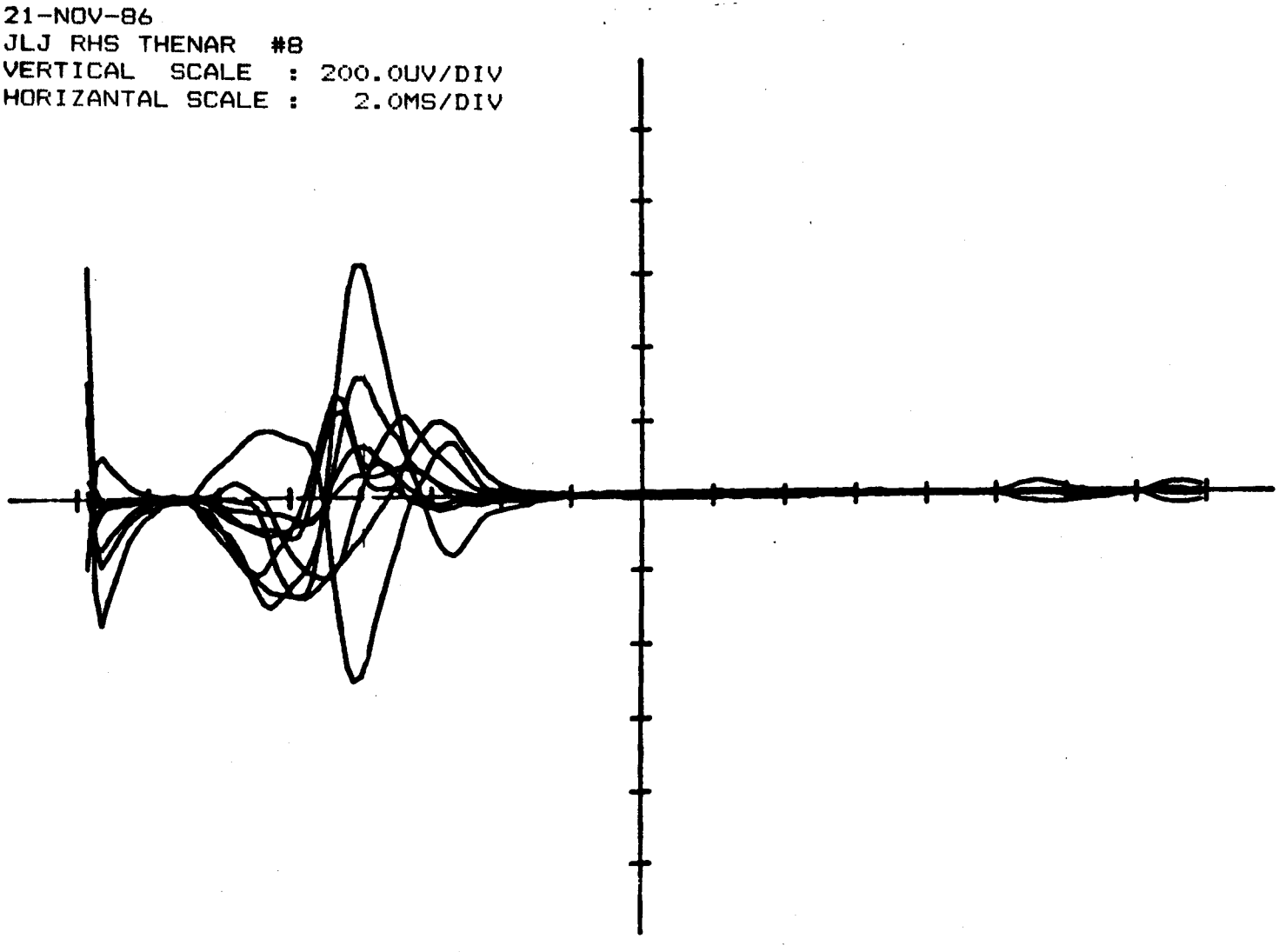


Figure 4-26 Recovered MUAPs - Ranking by Peak



In the regression method, the ranked position of a response is assumed to be one plus the number of MU's firing. Clearly, an incorrect ranking will result in incorrect extrapolation. The calculation of an "average" MUAP feature is obtained by taking the average of the MUAP features recovered from the ordered templates. If the order is incorrect, then the recovered MUAPs and features are altered radically, affecting the calculation of the "average" MUAP feature. This is illustrated in Figures 4-25 and 4-26 which display the MUAPs recovered using each feature for the badly behaved templates.

4.9.3. Discrimination of Responses

Each point in Figure 4-14 represents a response. These responses were assigned to a cluster based on the use of all of their one hundred time samples as discriminatory features. It would be convenient to use either area or amplitude as the discriminatory feature for responses. However, the overlap of the clusters as projected onto the X axis shows that two different clusters can have the same area. Therefore, area alone is an inadequate discriminant feature.

Figure 4-15 also demonstrates that area is not a sufficient feature to discriminate between increments in the response. If area were a sufficient feature, the relationship shown would be a step-wise function. It would be expected that area would remain constant as stimulus amplitude was increased until a new MU fired. Once the new MU had fired, area would remain constant with increasing stimulus amplitude until the next MU fired. Figure 4-15 does not show such a step-wise function, thus

confirming that area alone is an insufficient feature to discriminate between increments in the response. As discussed above, peak and area were directly correlated, and because of this, peak and area together would also be insufficient features to adequately discriminate the responses.

4.10. Estimated Number of Motor Units

4.10.1. The Relationship Between the Estimates

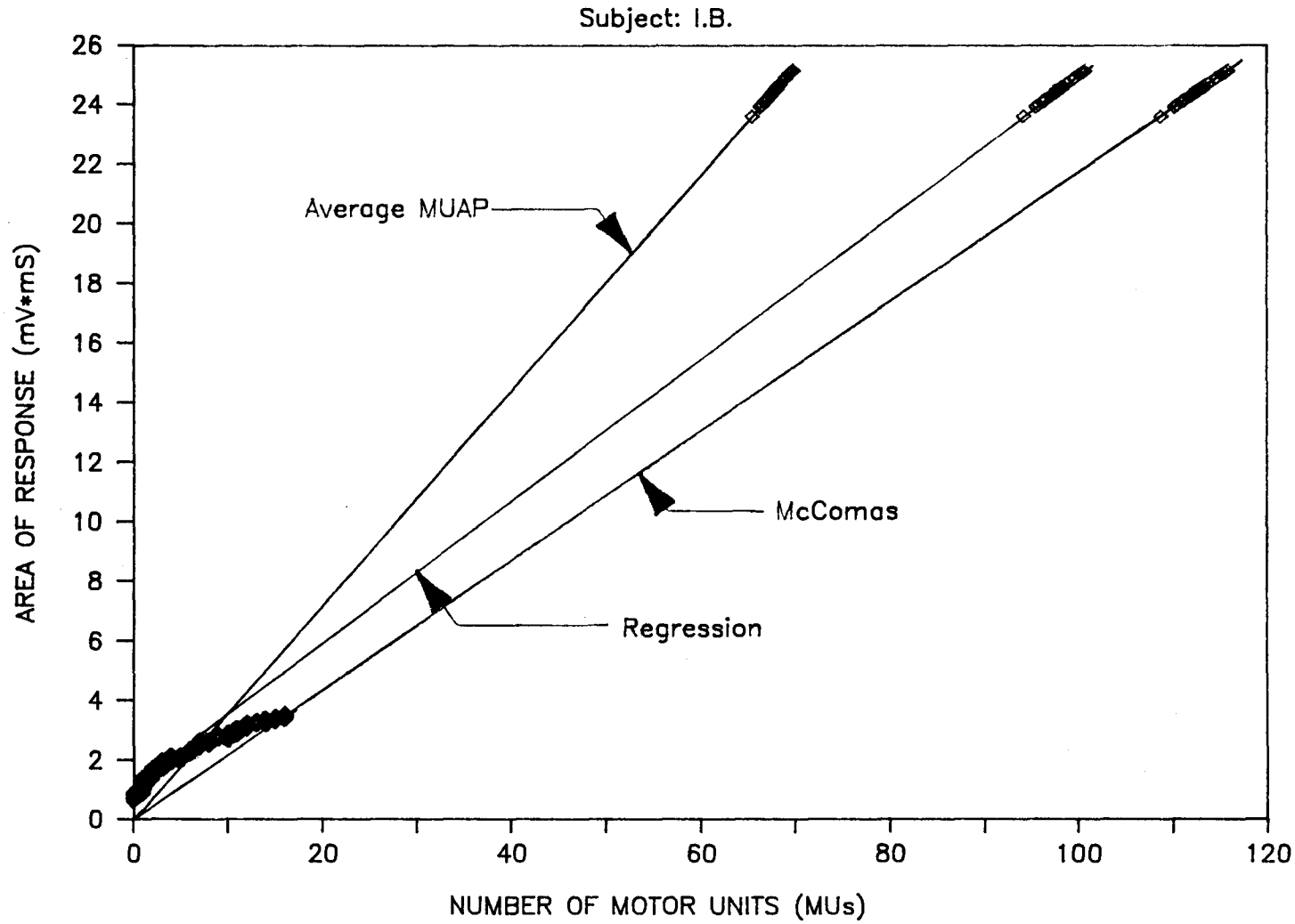
The automated motor unit counting system calculates six separate estimates, each with their own 95% confidence interval. There are three distinct methods used to obtain the estimates, the regression method, McComas' method and the Average MUAP method. Each of these methods is applied using two features, peak and area, for a total of six estimates as shown in Table 4-6. Where discrepancies exist between the estimates, only results from clinical trials will be able to determine which estimation method is the most accurate.

Table 4-6: MU Estimation Results.

Subject	AREA				PEAK				N
(Run#)	Regress.	McComas	Alt Ave	R^2	Regress.	McComas	Alt Ave	R^2	
JLJ A3	81 ± 9	81 ± 26	49 ± 25	0.95	16 ± 7	84 ± 23	17 ± 18	0.62	16
JLJ A4	113 ± 29	74 ± 27	53 ± 31	0.86	51 ± 18	94 ± 24	25 ± 20	0.74	11
JLJ A5	103 ± 27	105 ± 17	55 ± 23	0.79	22 ± 9	82 ± 17	14 ± 15	0.57	15
JLJ A6	55 ± 25	64 ± 4	48 ± 27	0.76	25 ± 14	66 ± 11	16 ± 9	0.66	7
JLJ A7	190 ± 19	164 ± 53	95 ± 38	0.96	101 ± 55	112 ± 33	41 ± 31	0.45	16
JLJ A8	72 ± 6	55 ± 17	42 ± 16	0.99	49 ± 34	62 ± 22	21 ± 14	0.50	9
LMC A1	132 ± 32	120 ± 38	59 ± 19	0.82	117 ± 46	145 ± 89	38 ± 33	0.62	16
JSL A1	148 ± 12	147 ± 43	129 ± 44	0.98	35 ± 19	104 ± 37	36 ± 43	0.52	11
IB A1	98 ± 17	113 ± 28	68 ± 43	0.89	32 ± 12	116 ± 46	26 ± 39	0.59	16
NPP A1	98 ± 16	170 ± 37	53 ± 29	0.93	30 ± 9	130 ± 56	18 ± 22	0.76	13
RCM A1	73 ± 9	117 ± 32	39 ± 20	0.94	16 ± 5	97 ± 29	9 ± 9	0.82	10
BJM A1	60 ± 7	74 ± 3	59 ± 34	0.97	49 ± 14	76 ± 16	33 ± 30	0.83	10
DBM A1	103 ± 26	102 ± 12	71 ± 43	0.95	35 ± 28	121 ± 22	22 ± 29	0.65	5
JGJ A1	224 ± 54	167 ± 73	90 ± 59	0.81	104 ± 116	130 ± 97	19 ± 43	0.15	17

The relationship which exists between the three methods can be described with reference to Figure 4-27. The methods all use linear extrapolation to estimate the number of motor units corresponding to the features measured from samples of the MEP. However, the methods are each based on particular equations of the line. The three methods would result in three identical estimates for data which lay on a straight line passing through the origin on the feature-vs-number-of-MUs plane.

Figure 4–27 Relation Between the Estimation Methods



4.10.2. The Regression Method

The accuracy of the regression method is dependent upon the degree to which the data points are described by a straight line fitted to the data points. The confidence interval given for the estimate shows an inverse relationship to the r^2 of the regression line and to the number of points used to generate that line. The data shown in Figure 4-27 suggest that a higher order polynomial or exponential function might be found which would better describe the relationship. The slope of the line has been biased by the greater density of points at the lower end of the range; this greater density of data points at the low end is not apparent in the figure.

4.10.3. The McComas Method

The McComas method is also based on linear extrapolation. However, the McComas line differs from the regression line because it is a two point extrapolation using the origin and the largest identified template. This method is expected to be compromised should the largest template point be an outlying point, unlike the average MUAP and regression methods which are based upon all of the data and are expected to provide a more robust estimate of the number of MU's. No data points lie on the origin. This demonstrates a source of error implicit in the automated version of McComas' method: zero MU's results in zero measured area. This error will be larger where the duration of the EMG response is short in proportion to the length of the window in which the statistics are calculated.

The confidence intervals assigned by McComas' method are directly related to the variance in the responses assigned to the maximum evoked potential and the variance in the responses assigned to the largest template.

4.10.4. The Average MUAP Method

Figure 4-27 shows the two point extrapolation using the origin and the area corresponding to the average MU found by decomposition of the templates. This third method assumes linearity, therefore the divergence of the estimate from this method from the estimates obtained by the other two methods is an indication of non-linearity of feature summation.

This chapter has presented the results obtained from tests of the automatic motor unit counting system. The importance of the system, its relevant features and its implications for further study are discussed in the next chapter.

CHAPTER 5.

CONCLUSION

The purpose of this research was to use a general purpose computer to implement the motor unit counting method developed by McComas. The resulting system was to be suitable for use in a research clinical environment, providing a flexible tool for clinical trials or for further development of the technique.

As a research tool, it was expected that the resulting automated system could be used to answer questions regarding nonlinear summation, bias in the recruitment of motor units, and the selection of features to be used for discriminating responses.

In the clinical environment, the system was required to have a well defined test protocol and minimal operator judgment requirements. This would result in a system which would provide reproducible results independent of operators and facilities. This demanded a system employing closed loop control of the stimulator for integrated data acquisition.

The automated motor unit counting system has been demonstrated to be a successful, functional working system by employing human subjects in preliminary trials. Implementation on a general purpose computer (PDP-11/34) in a high level language (FORTRAN) has resulted in a flexible system that is easily enhanced.

The motor unit counting system reduced the activities of the operator to monitoring the test progress and running the system. The software provides extensive user-friendly features. These include filtering of operator input for invalid responses, provision of default responses and disk file diagnostics. Furthermore, adaptive features of the system have eliminated the need for the operator to adjust the system for different subjects. All of the operator judgment and interpretation required in McComas' manual method was performed by the system; in order to accomplish this, the test procedure itself was analyzed and documented and the heuristic decision criteria were quantified.

It is important to note that the system remains similar to the manual version of McComas' method to allow a comparison of results between the manual and automated methods. This is expected to generate wider acceptance of the automated system. Also expected to foster acceptance are the estimate confidence intervals which are produced by the system to render the estimates more meaningful.

In the course of this thesis, additional observations were made:

1) Alternation was often the limiting factor in data acquisition and remains the most serious, unresolved problem with McComas' method. Alternation has a profound effect upon the reliability of the estimate, and no methods have yet been proposed to prevent its occurrence or accommodate its detrimental effects.

2) Nonlinear summation of the MUAP features has been suspected by other researchers as being a major shortcoming of the MU counting method. In this thesis, a number of indicators of the magnitude of this nonlinearity are presented.

3) Generalization from McComas' calculation formula have yielded two other methods of estimation. Of these two methods, estimation by extrapolation using regression promises to be a preferred method to McComas' method. This extrapolation uses all of the evoked signals acquired as opposed to the use of only those signals assigned to the largest template.

During any research project, many exciting directions for future work become apparent. Now that the system has been demonstrated on a small number of subjects, it must be used in clinical trials to obtain a comparison with the manual method and to obtain a database of expected ranges of estimates from normal subjects. These data will provide answers to the questions of bias in the excitation thresholds of the motor units and of the use of either peak, area or some other feature in the estimation calculation. This data can also be used to optimize the empirically assigned system parameters.

Several enhancements could be incorporated in the system. For example, persistent alternation should be detected and dealt with automatically. Now that the effects of non-linearity can be quantified, a system performing nonlinear extrapolation might reduce the associated error. Software control of the amplifier gain and stimulator range should be added to the system to eliminate manual adjustment of these controls. Increased memory would permit a larger number of templates, and should therefore decrease the uncertainty associated with the estimates. A post-data collection clustering of the evoked potential data would offer a method of establishing an optimum set of templates. This would minimize

the errors associated with incorrect assignment of signals which can occur with the ensemble average template definition used here.

Multi-channel recording may provide a means of distinguishing the response increments due to alternation from those due to genuine motor units. Alternate stimulation schemes should be explored as a means of reducing alternation, of obtaining a more representative sample of motor units, or of increasing the number of identifiable motor units. Some of these alternate stimulation schemes may include multiple stimulation sites or Wedenski inhibition.

In conclusion, this thesis represents a significant contribution to motor unit counting. Now that automatic estimation of the number of muscle motor units has been demonstrated, one can predict that motor unit counting will become a valuable and widely used tool.

* * *

APPENDIX

DESCRIPTION OF SOFTWARE

A.1. General Program Design

The main body of this thesis has described the rationale for the procedure used to estimate the number of motor units in a muscle. This appendix describes the details of the programming developed to implement this procedure. An overview of the system of programs is presented, and a detailed outline of each section of code is related to the underlying methods and assumptions. This appendix is intended for the reader who has understood the requirements of the system as presented in Chapter 3.

A.1.1. Implementation

The MU counting technique has been implemented on a PDP-11 computer as described in Chapter 3. The programs were run under the RT-11 (version 5.1) single job operating system. The Fortran programs were compiled with the PDP-11 FORTRAN-77 V5.0 compiler, the assembly language code was written using the MACRO-11 V5.01 assembler. To facilitate the implementation of the software on another computer system, the use of extensions to the ANSI language standard were minimized. Thus this system

can be easily moved to another facility with the following changes required:

a) Development of system-specific graphics routines;
b) Adaptation of the data file names to conform to the operating system requirements;
c) The development of machine specific routines for signal acquisition and time delay;
d) Modification of the console screen control characters if the terminal control is not compatible with the ANSI standard;
e) Adaptation of the printer control codes used to select and release the compressed printing mode of the printer.

A.1.2. Programming Philosophy

The design intent of this task was to produce a research system to be used in advancing the counting technique proposed by McComas. In line with this, the software has been written to facilitate modifications to the algorithm and the run-time parameters. The code is written to be easily followed - even where this may be at the expense of elegance or speed/space efficiency. The use of subroutines has been limited to code which: had to be written in machine language; was to be used extensively through out the programs; or which had been previously written and tested by others. The programs have been broken into subsections which are logically distinct units. These subsections pass data and parameters to each other using disk files rather than alternate methods such as chaining or COMMON blocks to facilitate manipulation of the data and parameters during testing of the modules. All code listings include extensive

comments with variable names being selected for their mnemonic qualities. The contents of all IF-statements and DO-loops are indented to enhance readability.

The automated system is broken into four distinct but related programs. They are executed in the order presented here:

1) INITL .FOR * Determine parameters, estimate motor threshold.
2) AVMUAP.FOR * Acquire EMG data for estimation of average MUAP.
3) MAXEP .FOR * Acquire EMG data for estimation of MEP.
4) PROCES.FOR - Estimate the motor unit count from the data.

The first three programs acquire data from the subject. Therefore, the programs marked with an asterisk should be executed in as short a time as possible to minimize shifts in the electrode positions or impedances. Such changes will result in errors in the final MU count estimate. The remaining program performs processing on signals previously acquired and stored. During a clinical trial, these four programs could be redefined as subroutines and chained together by creating a master program which would call each subroutine in succession. An overlay structure can be used to reduce the memory requirements. The four programs have the following functions:

INITL.FOR

The run-time parameters used during the previous test are stored in the file "RESPON.DAT"; the operator is able to select new parameters, thereby changing the run conditions, or use the previously defined (default) parameters. Other parameters, which are less likely to require manipulation by the operator, are initialized using DATA statements so

that the operator is not confronted with too many options. Finally, the stimulus threshold for the first MUAP is estimated. This is accomplished by employing feedback from the subject to determine the sensory threshold, and using this value as an estimate of the motor threshold. The response evoked at this stimulus level is displayed on the graphics screen. The operator can thus confirm that the stimulus is sub-threshold.

AVMUAP.FOR

This program segment acquires the EMG signals required to estimate the average motor unit response. The stimulus is slowly increased from just below the motor threshold until a number of motor units have been recruited. After each stimulus, the background noise is assessed; if found to be excessive, the sample is rejected. If the noise is within limits, then the response is accepted for processing. Various statistics and features are calculated and displayed. To decrease the effects of the stimulus artifact upon the statistics, an estimate of the stimulus artifact is removed from the response before it is displayed or processed and the period containing the bulk of the artifact is exempted from the statistical calculations. The signals undergo clustering to identify each significant change in the response which is presumed to result from a change in the identity of the motor units firing. The clustering is accomplished using the time domain signal samples as features, and the euclidean distance as a measure of the degree of fit to the templates used as cluster centers. New templates are created when the response to a constant stimulus is consistently different from any existing template. The permissible difference is established by considering the pre-stimulus

noise samples. The templates are constantly updated to reflect the average of all the responses assigned to them. These templates and their statistics are stored in disk files named "TMPLT1.DAT" and "STATS1.DAT" at the completion of the acquisition. All of the EMG signals accepted for processing are stored in the file "EMG.DAT". Because of memory limitations, there is a limit to the number of templates which can be supported at one time. When the available template positions are close to being filled, the spuriously generated templates, those which were formed by chance because of noise, are identified and their template positions made available once again. Execution is discontinued when all of the template positions have been used. The templates are displayed on the graphics screen.

MAXEP.FOR

The maximum evoked potential (MEP) is identified by this program. The stimulus required to evoke the MEP is the stimulus beyond which the EMG response does not increase. To prevent potentially long painful trains of stimuli, the operator is required to press the <return> key for each pulse. The stimulus level is increased from the threshold stimulus in large steps until the percentage increase in the response is less than a preset limit. Samples of the MEP are acquired and stored in the disk file "MEP.DAT"; statistics are calculated and stored in the file "STATS2.DAT" for use in the subsequent calculation of the number of motor units. As in AVMUAP.FOR, signal samples are rejected if the background noise is excessive. The MEP signals and their statistics are displayed permitting the operator to monitor the progress of the test. The ensemble average of the MEP signals is displayed along with corresponding statistics.

PROCES.FOR

This routine extracts an estimate of the average MUAP from the templates generated by AVMUAP.FOR. This estimate, along with the MEP data generated by MAXEP.FOR, is used to calculate a measure of the number of motor units present in the muscle under test.

A.2. Data File Descriptions

A description of the data files used to pass information between the program segments will aid in understanding the structure and function of this motor unit counting system.

File : RESPON.DAT Unit #1 (from INITL.FOR)
Type : Sequential, formatted

Variable	Format	Description
TITLE(50)	50A	Descriptive file header
DDMMYY(9)	9A	Date of the test
ZERO	I4	Effective zero point for the stimulator
PREAMP	F9.4	Gain of the preamplifier
HCOPY	I3	A flag indicating hard copy required during test
SRATE	I4	Sampling rate in Hz
NN	I4	Number of signal samples stored
GAIN	F4.1	Analog gain setting
PAUSE	I5	Additional inter-stimulus interval in mS
INCR	I3	Size of stimulus increments (bits)
SETTLE	I4	Number of stimulus artifact lock-out bits
ST(25)	25F9.4	Some empirical limits
WINDOW	I3	Number of samples considered during processing
STHRESH	I4	Motor threshold stimulus

File : FTR.DAT Unit #2 (from AVMUAP.FOR)
Type : Sequential, formatted

Variable	Format	Description
TITLE(50)	50A	Descriptive file header
DDMMYY(9)	9A	Date of the test
D	I2	Number of features per signal
.	.	.
.	.	.
.	.	(for each signal acquired...)
S*SMULT	I4	Stimulus value for the signal
CLUSTR	I2	Template to which the signal was assigned
FEATR(D)	dF11.5	The "D" features calculated for the signal
.	.	.
.	.	.
.	.	(to the last signal...)
'END'	A3	End-of-data marker

File : EMG.DAT Unit #3 (from AVMUAP.FOR)
Type : Direct access, RECL=1

ASV	Variable	Description
1-50	TITLE(50)	Descriptive file header
51-59	DDMMYY(9)	Date of the test
60	NN	Number of samples per signal
61	NSAMPL	Number of signals sampled
62	INT(GAIN*10)	
.	.	.
.	.	(for each signal "I"...)
XX	S*SMULT	Stimulus value for the I^{th} signal
(XX+1)-	SIG(NN)	The NN samples of the I^{th} signal
.	.	(with XX = (I-1)*(NN+1) + 63)
.	.	.
.	.	(to the $NSAMPL^{th}$ signal)

File : TMPLT1.DAT Unit #4 (from AVMUAP.FOR)
Type : Direct access, RECL = 2

ASV	Variable	Description
1-50	TITLE(50)	Descriptive file header
51-59	DDMMYY(9)	Date of the test
60	NN	Number of samples per signal
61	T	The number of templates in the file
.	.	.
.	.	.
.	.	(for each template "I"...)
YY	"I"	Template number
YY+1	M	Number of members in the I^{th} template
(YY+2)-	TEMPLT(NN)	The NN samples of the I^{th} template
.	.	(with YY = (I-1)*(NN+2) + 62)
.	.	.
.	.	(to the T^{th} template...)

File : MEP.DAT Unit #8 (from MAXEP.FOR)
Type : Direct access, RECL = 1

ASV	Variable	Description
1-50	TITLE(50)	Descriptive file header
51-59	DDMMYY(9)	Date of the test
60	NN	Number of samples per signal
61	NSIG	Number of MEP signals sampled
62	INT(GAIN*10)	
.	.	.
.	.	(for each signal "I"...)
ZZ	S*SMULT	Stimulus value for the I^{th} signal
(ZZ+1)-	SIG(NN)	The NN samples of the I^{th} signal
.	.	(with ZZ = (I-1)*(NN+1) + 63)
.	.	.
.	.	(to the $NSIG^{th}$ signal)
WW	TMPLT(NN)	The NN samples of the ave of the NSIG signals
		(with WW = NSIG*(NN+1) + 63)

File : STATS1.DAT Unit #10 (from AVMUAP.FOR)
Type : Sequential, formatted

Variable	Format	Description
TITLE(50)	50A	Descriptive file header
DDMMYY(9)	9A	Date of the test
D	I2	Number of features per signal
T	I2	Number of templates
.	.	.
.	.	.
.	.	(for each template "I"...)
"I"	I2	Template number
M	I3	Number of signals averaged to form the template
SMIN	I5	Minimum stimulus with response grouped in "I"
SMAX	I5	Maximum stimulus with response grouped in "I"
.	.	.
.	.	.
.	.	(for each of the D features...)
AVE(I,J)	F10.5	Average of feature "J" for I^{th} template
SIGMA(I,J)	F10.5	Standard deviation in dimension "J"
R(I,J)	F10.5	Coefficient of correlation between the stimulus and the J^{th} feature
.	.	.
.	.	.
.	.	(...to the D^{th} feature)
.	.	.
.	.	.
.	.	(to the T^{th} template)

File : STATS2.DAT Unit #11 (from MAXEP.FOR)
Type : Sequential, formatted

Variable	Format	Description
TITLE(50)	50A	Descriptive file header
DDMMYY(9)	9A	Date of the test
D	I2	Number of features per signal
.	.	.
.	.	.
.	.	(for each signal acquired...)
S*SMULT	I4	Stimulus value for the signal
FEATR(D)	dF11.5	The "D" features calculated for the signal
.	.	.
.	.	.
.	.	(to the last signal...)
'END'	A3	End-of-data marker
NU	I2	Number of MEP signals sampled
SMIN	I4	Maximum stimulus used to evoke the MEP
SMAX	I4	Minimum stimulus used to evoke the MEP
.	.	.
.	.	.
.	.	(for each of the D features...)
AVE(J)	F10.5	Average of feature "J"
SIGMA(J)	F10.5	Standard deviation in dimension "J"
R(J)	F10.5	Coefficient of correlation between the stimulus and the J^{th} feature
.	.	.
.	.	.
.	.	(...to the D^{th} feature)

A.3. Program Descriptions

After completing this brief overview of the motor unit counting system, a more detailed description of the four member programs is now presented. This is followed with a description of the subroutines used.

A.3.1. INITL.FOR

This first program functions to provide a central location for defining various non-interactive parameters which are used to control the test procedure. The operator is prompted for a character string which is used along with the current date obtained from RT-11 to identify all of the plots, print-outs, and disk files generated during the test. The operator is presented with three options regarding the routing of the hard copy output which will be produced, the simplest being that the line printer receives the output immediately. However, to reduce program execution time and the audible noise level, the default is for the output to be sent to a disk file (PRNTRx.DAT[3]) which can be printed at a more favorable time. Alternatively, the operator can elect to have no record of the test produced.

The operator can elect to change the run-time parameters; to use the default parameters from RESPON.DAT; or to abort the test. The file RESPON.DAT must already exist with the records correctly formatted. This can be accomplished using an editor should the file accidentally be

---

[3] AVMUAP.FOR produces PRNTR1.DAT, and MAXEP.FOR produces PRNTR2.DAT

deleted. If the operator chooses to enter new parameter values, he is then prompted for each of seven values. Whether the values come from the keyboard or the RESPON.DAT file, they are checked to ensure that they are within a reasonable range. If not, the READ statement is repeated. The parameters are then displayed on the console screen.

To determine the sensory threshold, the subject is continuously stimulated and the response recorded. The stimulus is stepped up slowly from zero volts until the Schmitt trigger is pressed or the maximum stimulus is reached, which will be 100V when the "OUTPUT MULTIPLIER" control set to "X1". The step size used is the value of parameter "INCR" which has a typical value of 3 (3/1900*100V = 0.16V). The stimuli are delivered at a rate of approximately nine per second (9Hz), requiring 70 seconds to reach the maximum stimulus (100V/.16V/9Hz). If the maximum stimulus is reached, the stimulation terminates and the operator is prompted to check the set-up and press <return> to restart the stimulation from zero. If the Schmitt trigger is pressed before the maximum stimulus is reached, the value of the last stimulus is displayed on the console, and the response to that stimulus is displayed on the graphics screen. The signal is displayed by the subroutine SCOPE.FOR which is discussed later.

The operator can view the plot of the response to this stimulus and choose one of four options: He can confirm that the response was subthreshold and (the default) restart the stimulation loop, or (Q) quit the program. Alternately, the entire program can be restarted (R) - including the selection of the parameters; or, if a MU response is seen on the plot of the response and is to be ignored, (N) the stimulation loop can be re-

entered. If the operator chooses to restart the stimulation loop, he is asked whether the graphics screen is to be cleared before the next plot. The number of plots since the last erase is recorded, and sufficient line-feeds are sent to the graphics screen before writing the stimulus value to prevent overwriting the previous value. When the operator chooses to quit the program, the RESPON.DAT file is updated with the current parameter values and the average stimulus value of those trials which resulted in sub-threshold responses. The time of day (from RT-11) is recorded on the graphics screen.

A.3.2. AVMUAP.FOR

The role of this program is to obtain sufficient data to enable further processing to estimate the average motor unit response. To achieve this goal, an adequate number of representative motor units must be recruited and sufficient samples of the compound potential responses are required.

When executed, AVMUAP.FOR first reads the run-time parameters from the RESPON.DAT file and then displays them. If the HCOPY flag is set, the parameter values are sent to the printer (or to the PRNTR1.DAT disk file), followed by a header for the table of calculated statistics to be produced. The data files for the EMG samples and the calculated features are OPENed and various counters, accumulators, and limits, are initialized. The operator is presented with a menu of options:

<Q> Quit - Exit the acquisition loop; exit program if no data has accumulated.

<R> Reset - all arrays and variables are reinit-

ialized and the program execution restarted from the beginning.

<G> Gain reset - The arrays and variables are re-initialized, and the test restarted with a new analog processor gain.

<S> Stimulus intensity reset - The next stimulus value to be used can be set. Return to this menu.

<F> Faster acquisition - The display flag is set "FALSE" to forgo the slow, signal-by-signal, display on the Tektronix graphics terminal. Return to this menu.

<A> Automatic creation of new templates. The AUTO flag is set "TRUE"; this makes the acceptance of new templates automatic. Return to this menu.

<D> Display flag is set "TRUE", AUTO flag is set "FALSE". Result is the display of each signal that is accepted for processing, and the automatic formation of new templates is disabled. Return to this menu.

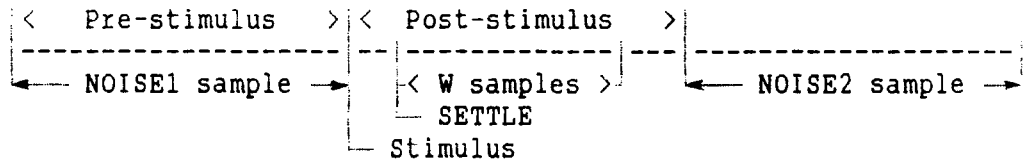
< > default - [Re]enter the data acquisition/processing loop.

The operator is also prompted to set the ERASE flag which selects the manner in which the Tektronix screen is to be erased: (Yes, No, or Automatically before each plot).

The program flow enters a loop in which the subject is stimulated and the EMG responses are displayed and processed. The loop is exited by pressing the Schmitt trigger switch; by reaching the maximum stimulus amplitude; by requiring more template positions than available; or by filling the available templates with a sufficient number of signal samples. A time delay provided by WAIT.MAC provides an upper limit to the frequency of stimulation. The Schmitt trigger is checked just prior to stimulation and acquisition. If it has been pressed then the loop is interrupted and the operator returns to the menu discussed previously.

The subroutine AQUIRE.MAC is invoked which triggers the stimulator and returns the time samples of the pre- and post-stimulus EMG as shown in Figure A-1. The operation of this routine is discussed in more detail later.

Figure A-1 : Time Division of the Signal



Once the signal has been acquired, it is checked for saturation at the preamp or the A/D converter:

$$^1/_3*4095 < \text{MEAN1} < {}^2/_3*4095, \quad \text{and,}$$

$$1 < \text{SIG(I)} < 4095, \quad \text{for I: SETTLE+1} \leq \text{I} \leq \text{SETTLE+W}$$

If either of the above conditions is not met, the signal is discarded and a new signal obtained. The average of the two means returned by AQUIRE.MAC, that is, (MEAN1+MEAN2)/2, is subtracted from all of the signals to remove the effects of any DC offset. The signals are left in integer format to reduce memory requirements; the time sample values being related to the input signal by two calibration factors.

A time calibration factor (F_T) is obtained from:

$$\text{Time duration (mS)} = \frac{\text{(\# of samples)}}{\text{(sampling rate)}} * 1000 \frac{\text{mS}}{\text{S*Hz}}$$

Giving : $F_T = 1000/(\text{Sampling rate})$ mS/sample

The amplitude calibration factor (F_A) is given by:

$$\text{Input voltage (mV)} = \frac{10}{4096} * \frac{1000}{\text{(Preamp gain)*(Amp gain)}} \frac{\text{V*mV}}{\text{bits*V}}$$

Therefore, $F_A = 2.441/(\text{Total gain})$ mV/bit

A number of statistics are calculated for the first and third signal periods (NOISE1 & NOISE2, Figure A-1), to be used in assessing the level of background noise and in calculating the decision criterion used during template matching. The statistics are calculated using all NN time samples in each of the two periods. They are:

a) The average of the rectified signal samples, Nsig ave;

b) The variance of the rectified signal samples, Nsig var;

c) The area under the rectified noise signal curve, Nsig area;

d) The amount of baseline drift between the noise periods:

$$\text{Drift} = |\text{Mean1} - \text{Mean2}| * F_A / (\text{Nsig1 var})^{1/2}$$

If the noise signal sample average or standard deviation for either of the two noise signal periods is in excess of empirical limits, the signals is discarded and program flow returns to the beginning of the acquisition loop to acquire a new EMG signal.

When the background noise has been found to be within the acceptable limits, statistics to be used in assigning the evoked potential to a template are obtained. These statistics are calculated over all of the samples of the second signal period which lie within a window W samples wide, that is:

the samples "Sig(i)", for SETTLE+1 $\leq$ i $\leq$ SETTLE+W;

The statistics are:

a) The area under the rectified signal curve:
Sig area = (Sum of |sample|) * F_T * F_A

b) The peak sample of the period:
Sig peak = (Maximum of samples) * F_A

c) The time from stimulus until the peak found in b):
Latency = (The sample # where peak occurs) * F_T

d) The peak-to-peak amplitude of the signal:
PPamp = [(Maximum sample) - (Minimum sample)] * F_A

e) The amplitude of the rectified signal 4mS after the peak of the signal occurred:
Sig4 = |Sample @ (Latency + 4)/F_T|

If the DSPLY flag is set, the samples from the first and second signal periods are displayed on the graphics screen (using SCOPE.FOR), while the noise statistics and the signal features are written to the console screen. Since the display routine is slow (3 mS per vector) only every second sample is displayed. This will normally result in a smooth looking plot, and yet cuts the plotting time in half. If this is the first signal to be processed, there will be no templates to compare the signal with. The MATCH flag is cleared and then the program flow skips ahead to a section of code discussed later where a template can be creat-

ed. With subsequent signals, templates will exist, and the evoked signal is now compared with all existing templates and the degree of match is assessed.

Because of memory limitations, there are Q array positions available for templates. It is possible for a template position to be recycled as explained later. If a template is no longer required, its position in the various arrays may be declared "free" and the next template created will take its place. The array **A** is used to keep a record of the template # addressed by each array index. For example, the second template to be created will use the second array position, thus **A**(2) = 2. If this template is later eliminated, then the second position may then be used by the next template to be formed, say, template # 18, and **A**(2) set to 18. The variable "NEXT" holds the number of the first empty (unused) array position while "QQ" is the next template number to be used. These variables change values as the templates are created and discarded, and the array **A** provides the flexibility for this dynamic use of the template array.

The first template will always be created in array position #1 and will always consist of the sub-threshold signal; that is, the background EMG and the stimulus artifact, but no MUAP activity. This template is used to estimate the artifact contributing to the signals acquired so that the artifact does not affect the template matching described in the next paragraph. The estimated artifact (A_E) contribution to a sample of a signal evoked by a stimulus of amplitude S is:

$$A_E = \text{Template} * S/S_1 / F_A$$

Where S_1 is the sum of the N_1 stimulus amplitudes used to obtain the N_1 signals summed into template #1. In this manner, an estimate of the stimulus artifact is removed from the signals collected before they are added into the template to which they are assigned. The exception is template #1 which does not, of course, have the artifact removed from its component signals.

The samples of the second period, Sig(i), which are within the defined window W, are compared with the corresponding samples of each of the existing templates, Tmp(i). The number of the template which matches the signal most closely is recorded. The statistics defining the degree of matching are: the area between the template and the signal (AREA), and the RMS value of the differences between the two curves (DIFF), as follows:

$$AREA = \left(\sum_{i=SETTLE+1}^{SETTLE+W} \left| Sig(i) - *A_E - Tmp(i) \right| \right) * F_T * F_A$$

and,

$$DIFF = \left(\sum_{i=SETTLE+1}^{SETTLE+W} (Sig(i) - *A_E - Tmp(i))^2 \right)^{1/2} * F_A$$

*Except when testing against template #1, the estimated artifact, A_E, is subtracted from the signal.

*After the signal has been compared with each of the templates, CA and CD will contain the best matching template numbers by AREA and DIFF respectively.

The signal will be assigned to the best fitting template if all of the following criterion are satisfied:

a) CA = CD; that is both measures indicate the same template.

b) AREA/(Nsig area) $\leq$ (an empirical limit)

c) DIST/(Nsig var)/W $\leq$ (an empirical limit)

d) This is not the first signal to be assigned.

If one or more of these criteria are not satisfied, a decision is made whether to create a new template or to ignore the signal - thereby ruling that the lack of match was due to the stochastic nature of the signal. If one or more of the above four criteria are not satisfied, the MATCH flag is cleared and the signal is added to a "SAVE" bin which holds the sum of consecutively acquired ill-fitting signals. Another bin, "DIRT", is the sum of the differences between consecutive ill-fitting signals, and their best fitting templates:

$$\text{Save} = \text{Save} + (\text{Sig} - {}^{*}A_E)$$

$$\text{Dirt} = \text{Dirt} + (\text{Sig} - {}^{*}A_E - [\text{template}\ /N_T\ /F_A])$$

*Except when the best fitting template is template #1, the estimated artifact, A_E, is subtracted from the signal.

If less than the required number of consecutive "misses" have occurred, then the program returns to get another response at the same stimulus level. If enough (= NM) misses have occurred then the average of the differences recorded in DIRT is magnified and displayed on the graphics screen using SCOPE.FOR :

$$\text{Displayed signal} = 5 * \text{DIRT}/(\#\ \text{misses})$$

As in the case of the display of the whole signal, only the even numbered samples are displayed. If the DSPLY flag is set, then the magnified ensemble average of the differences is shifted downward to prevent this curve and the plot of the evoked signal from overlapping on the screen.

If the AUTO flag is set, and the number of existing templates is less than the number of template positions, the sum of the ensemble average of the signals forms a new template :

TEMPLATE(QQ) = SAVE/(# misses)

The variable QQ is incremented and processing continues as though a normal match had been detected. If there are no free template positions, the acquisition loop is exited. If the AUTO flag is clear, then the operator is prompted for the number of the template to which the ensemble average is to be assigned. The operator's input is checked to ensure that it lies in the range 1 through QQ. If it's outside this range, the program will jump back to acquire another signal as in the case where less than NM unmatched responses had been evoked. If the input is within this range, the template number is converted into an array index. If an attempt is made to start a new template before there are sufficient (= 5) samples in template #1 the program skips the operator back to the prompt for a new stimulus amplitude. The operator can assign the signal to a new template as would have happened had the AUTO flag been set, or he can assign it to an existing template.

Once the signal has been assigned to a template, the "SAVE" bin, "DIRT" bin, and NM (the number of times that no matching template was found) are all cleared. If the HCOPY flag is set, various signal statistics are sent to the printer. The templates and their statistics are updated to reflect the addition of the signal. The signal feature data are written to the FTR.DAT disk file and the signal samples are sent to the EMG.DAT file. The stimulus value (S_{NEXT}) to be used to acquire the

next signal is now calculated. If the number of templates currently defined (NT) is less than a target number of templates (TAT), the stimulus is incremented by the amount "INCR", or by 3*INCR if the last signal was assigned to a template already representing many signals :

that is,

$$S_{NEXT} = S + INCR, \quad \text{for } NT \leq TAT$$

$$S_{NEXT} = S + 3*INCR, \quad \text{for } NT > TAT$$

The new stimulus value is checked to ensure that it is less than the maximum, that is : $S + ZERO \leq 4095$. If it is too large, the operator is instructed to increase the "OUTPUT MULTIPLIER" control (of the stimulator) by one position. Both the stimulus and INCR values are rounded UP to the next even integer and halved. The variable SMULT records the current setting of the output multiplier. If SMULT has not exceeded 4 (the maximum setting on the stimulator used) program control returns to the beginning of the acquisition loop to get the next signal; other-wise the acquisition loop is exited.

If the number of templates in use (NT) exceeds the value of TAT, each template is examined to find those which represent too small a number of signals. If a template "i" has less than TA member signals, an

attempt is made to evoke additional members by re-stimulating with an appropriate stimulus value. The stimulus value used will be:

$$S_{NEXT} = \frac{(S_i max + S_i min)/2 + (U - 1/2) * (S_i max - S_i min)}{\text{(Output multiplier setting)}}$$

where,

$S_i max$ is the largest stimulus value which has evoked a signal assigned to template "i".
$S_i min$ is the smallest stimulus value which has evoked a signal assigned to template "i".
U is a number uniformly distributed in the range 0 - 1.

The number of attempts to fill template "i" with at least TA signals is recorded in the array TRY. When the value of TRY(i) reaches a large enough number (= TA) no further attempts will be made to evoke additional members into template "i". If TRY(i) has reached this limit without the template embodying a minimum number (= TINY) of members, the template position is released by clearing the various array elements which are used to record the data for that false template. When all existing templates either represent enough signals or have had enough attempts made to fill them, the target number of templates is increased by two (that is, TAT = TAT + 2). This increase in the number of possible templates can be repeated until the target number of templates exceeds the number of array positions, at which point the acquisition loop is exited.

When the acquisition loop has been exited the FTR.DAT and EMG.DAT files are closed. Each template having more than one member is displayed on the graphics screen and has its time samples saved in the TMPLT1.DAT disk file. The template statistics are calculated, displayed, written to the printer (if the HCOPY flag is set), and saved in the STATS1.DAT disk file. The statistics calculated for each template for each feature are:

the mean, the standard deviation, and the coefficient of determination between each feature and the stimulus.

A.3.3. MAXEP.FOR

This program determines the maximally evoked potential (MEP) required by PROCES.FOR to estimate the number of motor units. The procedure is to increase the stimulus until the evoked response fails to increase. Samples of the response at that stimulus intensity are averaged to obtain the estimate of the MEP. The EMG signals are stored in the disk file "MEP.DAT". The statistical features of the MEP samples are stored in the STATS2.DAT file. Because of the strong stimulus intensities involved, care is taken to ensure that the subject can not receive a long, painful train of stimuli.

The operator is prompted to set the stimulator "OUTPUT MULTIPLIER" control to the X4 position, and to forewarn the subject that this part of the test may offer some discomfort. The run-time parameters are retrieved from the RESPON.DAT disk file and displayed. The sample number - time calibration factor (F_T) is calculated as in AVMUAP.FOR. If the HCOPY flag is set, a header is sent to the printer which incorporates the character string passed from RESPON.DAT as well as the current time-of-day. The data files which will record the evoked responses and the statistical features, MEP.DAT and STATS2.DAT, are OPENed. The various arrays, variables and flags are initialized, and the operator prompted for the analog processor gain setting which is used to calculate the amplitude calibration factor F_A. A menu of options allows the operator to:

<R> Reset the program to the initial conditions
<G> Change the analog processor Gain setting
<Q> Quit the acquisition and process any data acquired
<S> Change the current Stimulus value
< > Default is to (re)enter the acquisition loop

Finally, the operator selects the erase mode for the Tektronix graphics screen: Erase, don't erase, or erase automatically before each plot.

The acquisition loop acquires the evoked response from the subject, processes it, displays it on the graphics screen and checks to see if the response has increased from the previous one. Before the subject is stimulated, a "PAUSE" statement requires a <Return> key-press; this ensures that a long uninterrupted string of stimuli can not occur. The Schmitt trigger is checked, and if it has been pressed the operator is returned to the menu discussed above. Only then is the AQUIRE.MAC subroutine invoked to sample the EMG data. The signal is tested for saturation which is suspected if either of two conditions is not met:

$$(^1/_3 * 4095) < \text{MEAN1} < (^2/_3 * 4095) \quad \text{and,}$$

$$1 < \text{SIG}(i) < 4095 , \quad \text{for } i: \text{SETTLE}+1 \leq i \leq \text{SETTLE}+W$$

If saturation is suspected, the signal is discarded and the acquisition process repeats; otherwise the pre-stimulus signal (NOISE1) average is subtracted from the signals to remove the DC offset.

Statistics are now calculated which will be used to decide if the background EMG activity is excessive. The statistics are calculated as in AVMUAP.FOR, but only for the NOISE1 samples. The statistics include the average (AVE_N) and variance (s^2_N) of the rectified samples, and the area

($AREA_N$) under the rectified NOISE1 signal curve. These statistics must meet the following conditions for the signal to be retained for processing:

$$AVE_N \leq \text{(an empirical limit) AND } AREA_N \leq W*F_A*F_T$$

$$s_N \leq \text{(an empirical limit) AND } s_N \leq 2*F_A$$

If the background EMG noise is within the specified bounds, the statistical features described for AVMUAP.FOR are calculated for the post-stimulus signal (SIG). The statistics are written to the console screen and the signal is displayed on the graphics screen.

If this is not the first signal to be acquired, the signal (SIG) is compared with the last response (SAVE) to detect any increase. Two statistics are calculated for this purpose: the average increase of the samples of the two signals (DIFF), and the area between the two curves (AREA):

$$DIFF = \sum_{SETTLE+1}^{SETTLE+W} [SIG(i) - SAVE(i)]^2 * F_A/W$$

and,

$$AREA = \sum_{SETTLE+1}^{SETTLE+W} |SIG(i) - SAVE(i)| * F_T * F_A$$

These statistics are displayed (and printed if the HCOPY flag is set). The MEP is declared to be found when two conditions are satisfied :

$$DIFF > 0$$

and,

$$AREA/FEATR(1) < \text{(An empirical limit)}$$

If the MEP has not been found, a copy of the evoked signal is retained and the stimulus is incremented by INCR. If the stimulus is at or beyond the maximum a warning message is displayed and the stimulus is set to the maximum value. The program then loops back to get the response to the new stimulus level.

Once the MEP is found, a total of N MEP samples are acquired with the stimulus updated using the following rule:

$$S_{NEW} = SC/SMULT + INCR*(U - 1/2)$$

with;

SC = Stimulus at which MEP was found
SMULT = Stimulator "OUTPUT MULTIPLIER" setting
U = A uniformly distributed # in the range (0 - 1)

Again, the stimulus value is checked to ensure that it does not exceed the maximum before looping back to get the next sample. The features and stimuli for each MEP are recorded. When N MEP samples have been sampled then the ensemble average is displayed on the graphics screen and the MEP statistics calculated. The statistics (the average, standard deviation, and coefficient of determination with the stimulus for each of the features) are displayed and written along with the MEP ensemble average and features to the disk files.

A.3.4. PROCES.FOR

This program uses the data acquired and processed in AVMUAP.FOR and MAXEP.FOR to calculate estimates of the number of viable motor units. The individual MU responses are extracted from the compound potentials and displayed. Various methods are used to obtain estimates, thereby permitting a comparison of the procedures.

In effect the program is executed twice; once using area as the governing feature; then using the peak amplitude. After the variables and arrays are initialized, the features in the STATS1.FOR file are ranked in ascending order. This is done by making two passes through the file; first reading the features into another array for in-place ordering using the Heapsort algorithm then building a table linking the features in the file with the elements in the ordered array. The ranking of the templates is displayed on the console screen. The next operation is to decompose the compound evoked potentials to obtain the MUAP signals which are summated to form them. To do this, the template samples stored in the TMPLT1.DAT file are successively (as ordered above) subtracted from each other:

$$MUAP = EMG_I/N_I - EMG_{I-1}/N_{I-1}$$

where;

MUAP is the response from the $(I-1)^{TH}$ MU
EMG_I is the compound response to the first $I-1$ MUs
N_I is the number of signals summed to form EMG_I
EMG_0 is a null response

The first template will consist of samples of the stimulus artifact only, and is skipped during the decomposition. The second template is assumed to represent the response to a single MU and does not require any decomposition.

The recovered MUAP is displayed on the graphics screen. The operator indicates whether the displayed signal represents a MU or just noise. If it is not accepted as a new MU, processing continues with the next template. If accepted, the MUAP is summed into an ensemble average, and a copy of the composite EMG signal is retained to be used in recovering the next MUAP. The area or peak feature is calculated for both the

composite EMG response and the recovered MUAP. The next template is then processed.

When the successive template subtraction is complete, the average values are calculated for a) the number of MUAPs per compound signal (AVEY), b) the feature values for the compound (AVEX), and c) the feature values for the recovered (AVEZ) signals. The mean and standard deviation of the MEP feature (MEPAVE & MEPSIG) are read from the STATS2.DAT file. Similar values (TOPAVE & TOPSIG) are read from the STATS1.DAT file for the largest compound signal which was successively decomposed into its individual MUAP signals.

A number of methods are used to obtain estimates for the number of active MUs. The first is to calculate the linear regression parameters from the 1 through N compound signal feature data, then use this linear model to extrapolate the number of units required to produce the MEPAVE. The equations for obtaining the N_1 estimate using this method are discussed in detail in Chapter 3.

The second method is that used by McComas - the feature, either area or peak, of the largest composite response (TOPAVE) is divided by the number of MUs (N) which produced the response. This gives an average MUAP feature. The number of MUs, N_2, is given by the ratio of the MEP feature (MEPAVE) to this average MUAP feature :

$$N_2 = \frac{\text{MEPAVE}}{\text{TOPAVE/N}} \pm \sqrt{\text{MEPSIG}^2 + \text{TOPSIG}^2}$$

The third estimation method is a variation on the second. The

features of the "N" individual MUAPs recovered from the composite responses, denoted **Z**, are used to calculate the third estimate :

$$FSIG = \frac{\sqrt{\sum_{i=1}^{N} (Z_i - AVEZ)^2}}{(N-1)}$$

Giving :

$$N_3 = \frac{MEPAVE}{AVEZ/N} \pm \sqrt{MEPSIG^2 + FSIG^2}$$

These three estimates are calculated and displayed using area as a feature, then using the peak of the signal. The coefficient of determination (r^2) for the size of the MUAPs and their order of recruitment is calculated and displayed :

$$r^2 = \frac{\sum_{i=1}^{N} [(Z_i - AVEZ) * (Y_i - AVEY)^2]}{\sum_{i=1}^{N} (Z_i - AVEZ)^2 * \sum_{i=1}^{N} (Y_i - AVEY)^2}$$

This completes the motor unit counting procedure.

A.3.5. AQUIRE.MAC

This assembly language program is used to trigger the stimulator and acquire samples of the EMG signals. Three consecutive segments of signal are returned; NOISE1, SIG, and NOISE2. The 12 volt trigger pulse for the stimulator is produced between the NOISE1 and the SIG signal

segments. All of the parameters passed to or from this subroutine are passed by address, and include:

To:	STIM	Stimulus pulse amplitude (parts per 4096)
	N	Number of samples per signal segment
	PERIOD	Period between signal samples in μS (inverse of sampling rate in MHz)
From:	NOISE1	First sample of the pre-stimulus signal
	SIG	First sample of the post-stimulus signal
	NOISE2	First sample of the last signal
	OFSET1	Average value of the NOISE1 samples
	OFSET2	Average value of the NOISE2 samples

Upon entering this subroutine, the control and status registers of the clock (CLKCSR) and the analog/digital (A/D) converter (ADCSR) are both cleared (except for the bit which denotes that the Schmitt trigger has been pressed, which is left unchanged). The first variable passed is the stimulus amplitude. The most significant 4 bits are set to 0000_2; this selects the first channel of the digital to analog (D-A) board which is on the digital output port of the LPS-11. The stimulus amplitude is then checked to ensure that it is greater than 2048, because the minimum voltage meaningful to the stimulator control input is 0 volts (= 2048, two's offset). If the value of STIM is less than 2048 then control returns to the calling program, otherwise the value is sent to the digital input/output (DIO) buffer.

The number of samples, N, is doubled to represent the number of words to be returned per signal. This value is added to the address of the first sample of each signal to calculate the address of the last sample; the beginning and end of each of the three buffers are now defined and stored. The two-word accumulators SUM1 and SUM2 are zeroed. They will be used to calculate the average of the N samples acquired for

the NOISE1 and NOISE2 signals. The stimulator trigger is set to the high state (= 2 V) by sending 14631_8 to the DIO buffer. This selects the second D-A channel and sets its output to :

$$\frac{(2457 - 2048)}{2048} * 10\ V = 2.0\ V$$

The clock is set to run at a rate of 1 MHz, counting up from 1 - PERIOD, overflowing, and resetting the count to 1 - period in a continuous cycle. The A/D converter is configured to sample the voltage on the channel #0 input each time the clock overflows.

With the hardware set up, the LPS-11 60 Hz line clock is polled until a zero crossing occurs, then the computer spins in a acquisition loop, polling the A/D "conversion complete" bit and moving the samples into the N1 buffer until the end of the buffer has been reached. As each sample is collected it is summed into SUM1. When the first buffer is full the output of the second D-A is set to -10 V by sending the value 10000_8 to the DIO buffer; the first 4 bits (0000_2) selecting channel #1. A software loop provides a delay of approximately 100 µS before the D-A output is returned to -2 V. The net result is a 100 µS -12 V pulse which meets the trigger pulse requirements of the Devices stimulator. Two more acquisition loops similar to the pre-trigger pulse loop discussed above fill the "S" and "N2" buffers with N samples each. The data sent to the N2 buffer is summed into the two-word accumulator "SUM2". Finally, the two sums SUM1 and SUM2 are divided by the number of samples "N" to form the means of the samples, and returned as OFSET1 and OFSET2 respectively.

The clock and A/D converter are disabled, and control returns to the calling program.

A.3.6. SCOPE.FOR

This subroutine displays data points on the Tektronix graphics screen. The routine first filters the input data for values outside a valid range; if so, an error message appears on the console, and execution stops.

If a LOGICAL flag is true then the screen is erased and the X- and Y-axis are plotted, and the legend re-written on the screen. The origin of the axes is positioned randomly about the centre of the display area to prevent needless damage to the screen phosphor. The legend consists of a descriptive header passed by the calling program, the current date, and the Y- and X-axis scales.

The data is scaled to occupy the largest screen area and have a axis scale which would be a multiple of 1, 2, or 5 units per centimetre. The data was also shifted to be centered on the screen.

A.3.7. SCHMIT.MAC

This subroutine returns a LOGICAL*1 flag set "true" if the schmitt trigger #1 threshold has been exceeded. The flag is passed by address and is immediately cleared (= 0) when this subroutine is invoked. If the most significant bit of the clock control/status register (CSR) is clear then the threshold has not been exceeded since the bit was last reset and control returns to the calling program. If the bit is set then it is cleared and the flag made "true" before returning.

A.3.8. WAIT.MAC

This subroutine waits the number of milliseconds specified by the single argument before returning to the calling program. The clock is stopped, then the argument value (passed by address) is checked: if it is less than or equal to zero then the subroutine is exited immediately. If greater than zero it is sent to the clock buffer and negated. The clock is started for a single cycle, counting at a 1 KHz rate. The clock control/status register is polled from within a wait loop until the clock count overflows. Control is then returned to the calling program. Overhead required for the call is less than 40 µS.

REFERENCES

Ballantyne, J.P. and S. Hansen. "A new method for the estimation of the number of motor units in a muscle. 1. Control subjects and patients with myasthenia gravis." *Journal of Neurology, Neurosurgery and Psychiatry*, 37, 1974a, 907-915.

Ballantyne, J.P. and S. Hansen. "Computer method for the analysis of evoked motor unit potentials. 1. Control subjects and patients with myasthenia gravis." *Journal of Neurology, Neurosurgery and Psychiatry*, 37, 1974b, 1187-1194.

Ballantyne, J.P. and S. Hansen. "New method for the estimation of the number of motor units in a muscle. 2. Duchenne, limb-girdle and fascioscalpulohumeral, and myotonic muscular dystrophies." *Journal of Neurology, Neurosurgery and Psychiatry*, 37, 1974c, 1195-1201.

Basmajian, J.V., H.C. Clifford, W.D. McLeod and H.N. Nunnally. Computers in electromyography. London: Buttersworths, 1975.

Brown, W.F. "A method for estimating the number of motor units in thenar muscles and the changes in motor unit counting with ageing." *Journal of Neurology, Neurosurgery and Psychiatry*, 35, 1972, 845-852.

Brown, W.F. "Thenar motor unit count estimates in the carpel tunnel syndrome." *Journal of Neurology, Neurosurgery and Psychiatry*, 36, 1973, 194-198.

Brown, W.F. and H.S. Milner-Brown. "Some electrical properties of motor units and their effects on the methods of estimating motor unit numbers." *Journal of Neurology, Neurosurgery and Psychiatry*, 39, 1976, 249-257.

Crago, P.E., P. Hunter Peckham, J.T. Mortimer and J.P. Van Der Meulen. "The Choice of Pulse Duration for Chronic Electrical Stimulation via Surface, Nerve and Intramuscular Electrodes." *IEEE Transactions on Biomedical Engineering*, 2, 1974, 252-264.

Delbeke, J. "Reliability of motor unit count in facial muscles." *Electromyogr. Clin. Neurophysiol.*, 22(4), 1982, 272-290.

De Luca, C.J. "Physiology and Mathematics of Myoelectric Signals." *IEEE Transactions of Biomedical Engineering*, 26 (6), 1979, 313-325.

Desmedt, J.E. New Developments in Electromyography and Clinical Neurophysiology. Karger: Basel, 1973.

Eisen, A., G. Karpati, S. Carpenter, and J. Danon. "The motor unit profile of the rat soleus in experimental myopathy and reinnervation." *Neurology (Minneapolis)*, 24, 1974, 878-884.

Feasby, T.E. and W.F. Brown. "Variation of motor unit size in the human extensor digitorum brevis and thenar muscles." *Journal of Neurology, Neurosurgery and Psychiatry*, 37, 1974, 916-926.

Feinstein, B., B. Lindegard, E. Nyman, and G. Wohlfart. "Morphologic studies of motor units in normal human muscles." *Acta Anatomica*, 23, 1955, 127-142.

Goodgold, J. and A. Eberstein. Electrodiagnosis of Neuromuscular Diseases, Third Edition. Baltimore: Williams & Wilkins, 1983, 79-84.

Gorman, P.H. and T. Mortimer. "The Effect of Stimulus Parameters on the Recruitment Characteristics of Direct Nerve Stimulation." *IEEE Transactions on Biomedical Engineering*, 30 (7), 1983, 407-413.

Guth, L. "An overview of motor unit structure & function." *Arch. Phys. Med. Rehabilitation*, 64 (9), 1981, 408-411.

Guyton, A.C. Human Physiology and Mechanisms of Disease. Philadelphia: W.B. Saunders Company, 1982.

Hansen, S. and J.P. Ballantyne. "A quantitative electrophysiological study of uraemic neuropathy. Diabetic and renal neuropathies compared." *Journal of Neurology, Neurosurgery and Psychiatry*, 41, 1978, 128-134.

Holman, J.P. Experimental Methods for Engineers. New York: McGraw-Hill Inc., 1978.

Hollinshead, T. Functional Anatomy of the Limbs and Back. Philadelphia: W.B. Saunders Company, 1976.

Jennekens, F.G., B.E. Tomlinson and J.N. Walton. "The extensor digitorum brevis: histological and histochemical aspects." *Journal of Neurology, Neurosurgery and Psychiatry*, 35, 1972, 124-132.

Kadrie, H.A., S.K. Yates, H.S. Milner-Brown and W.F. Brown. "Multiple point electrical stimulation of ulnar and median nerves." *Journal of Neurology, Neurosurgery and Psychiatry*, 39, 1976, 973-985.

Katz, Bernard. Nerve, Muscle and Synapse. Toronto: McGraw-Hill Inc., 1966.

McComas, A.J., P.R.W. Fawcett, M.J. Campbell, and R.E.P. Sica. "Electrophysiological estimation of the number of motor units within a human muscle." *Journal of Neurology, Neurosurgery and Psychiatry*, 34, 1971a, 121-131.

McComas, A.J., R.E.P. Sica, M.J. Campbell, and A.R.M. Upton. "Functional compensation in partially denervated muscles." *Journal of Neurology, Neurosurgery and Psychiatry*, 34, 1971b, 453-460.

McComas, A.J., R.E.P. Sica, and M.J. Campbell. " "SICK" MOTONEURONES A Unifying Concept of Muscle disease." *Lancet*, 1971c, 321-325.

McComas, A.J., R.E.P. Sica, A.R.M. Upton and F.Petito. "Myopathies: The neurogenic hypothesis." *Lancet*, 2, 1974d, 42.

McComas, A.J. Neuromuscular Function and Disorders. London: Butterworths, 1977.

Milner-Brown, H.S. and W.F. Brown. "New methods of estimating the number of motor units in a muscle." *Journal of Neurology, Neurosurgery and Psychiatry*, 39, 1976, 258-265.

Panayiotopoulous, C.P., S. Scarpalezos and T. Papapetropoulos. "Electrophysiologic estimation of motor units in Duchenne muscular dystrophy." *Journal of the Neurological Sciences*, 23, 1974, 89-98.

Panayiotopoulos, C.P. and S. Scarpalezos. "Electrophysiological estimation of motor units in limb-girdle muscular dystrophy and chronic spinal muscular atrophy." *Journal of the Neurological Sciences*, 24, 1975, 95-107.

Parry, D.J., G.W. Mainwood and T. Chan. "The Relationship Between Surface Potentials and the Number of Active Motor Units." *Journal of the Neurology Sciences*, 33, 1977, 283-296.

Peyronnard, J.M. "An experimental evaluation of the motor unit counting technique." *Canadian Journal of Neurological Sciences*, 2, 1975, 332.

Rose, A.L. and R.G. Willison. "Quantitative electromyography using automatic analysis: studies in healthy subjects and patients with primary muscle disease." *Journal of Neurology, Neurosurgery and Psychiatry*, 30, 1967, 403-410.

Scarpalezos, S. and C.P. Panayiotopoulos. "Myopathy or neuropathy in thyrotoxicosis." *The New England Journal of Medicine*, 289, 1973a, 918-919.

Scarpalezos, S. and C.P. Panayiotopoulos. "Duchenne muscular dystrophy: reservations to the neurogenic hypothesis." *Lancet*, 2, 1973b, 458.

Sica, R.E.P., A.J. McComas, A.R.M. Upton and D. Longmire. "Motor unit estimation in small muscles of the hand." *Journal of Neurology, Neurosurgery and Psychiatry*, 1974, 37, 55-67.

Shine, G. Unpublished internal report for the Department of Clinical Neurosciences. Hamilton, Ontario: McMaster University, 1982.

Sica, R.E.P., A.J. McComas, A.R.M. Upton and D. Longmire. "Motor unit estimation in small muscles of the hand." *Journal of Neurology, Neurosurgery and Psychiatry*, 37, 1974, 55-67.

Tanner, J.A. "Reversible Blocking of Nerve Conduction by Alternating-Current Excitation." *Nature*, 195, 1962, 712-713.

Vodovnik, L., C. Long,, E. Regenos, and A. Lippay. "Pain response to different tetanizing currents." *Arch. Phys. Med. Rehab.*, 46, 1965, 187-192.

Webster, John G. Medical Instrumentation: Application and Design. Boston: Houghton Mifflin Company, 1978.

Wiederholt, W.C. " "End-plate noise" in electromyography." *Neurology*, 20, 1970, 214-224.