MOTOR UNIT NUMBER ESTIMATES

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Motor Unit Number Estimates
An AANEM Demonstration

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INTRODUCTION

Damage to either the anterior horn cell or its peripheral axon is the essence of a neurogenic process, even though the associated change in muscle may be more apparent clinically. Diseases of motor neurons may produce histologic changes or physiologic changes without histopathologic correlates. An example of a physiologic abnormality is the irritability with excessive activity in the motor nerve seen in fasciculation. Physiologic disorders may cause a loss of function (as in conduction block) or no change in function (as in slowing of conduction). Degenerative or destructive processes result in the loss of an entire motor neuron or peripheral axon. Loss of motor axons or neurons and conduction block are the bases of the weakness found in most patients with neurogenic diseases. The severity of the clinical deficit is related directly to the number of motor neurons or axons (or both) that are lost or blocked. Therefore, an important part of the assessment of neuromuscular disease is determining the number of functioning motor units. It would be ideal to have an actual measure of the number of motor units, but current methods—physiologic, histologic, clinical, histopathologic—are not able to provide that number. Only electrophysiologic methods can be used to estimate the number of motor units in a muscle. Number of motor units refers to the number of functioning motor neurons or functioning motor axons innervating a muscle or group of muscles. A physiologic determination of the number of motor units is called motor unit number estimate (MUNE).

At best, histopathologic and anatomical determinations of the number of motor units in a muscle are only estimates. Two such studies have attempted to measure the number of motor units in human fetal and newborn tissue. Although the results of the two studies were similar, the values were sufficiently different to preclude the designation of a true standard measurement of the number of motor units innervating individual human muscles. However, these studies serve as a baseline comparison for the physiologic methods that have been developed for MUNE. The absence of a standard makes the direct comparison of the values obtained by different methods an equally important part of the assessment of the validity of individual methods of MUNE.

Standard diagnostic clinical EMG has always included a subjective estimate of the number of motor units in a muscle. Electromyographers have used recruitment analysis or interference pattern analysis (or both) with voluntarily activated EMG recordings to judge the number of motor units in a muscle. The EMG recorded with either surface or intramuscular electrodes during strong voluntary contractions summates the activity of the muscle fibers in the activated motor units to produce an interference pattern. The greater the number of motor units that are activated, the greater the density of the EMG pattern. Various measures of density, whether from subjective or automated methods, have been used to make judgments about the loss of motor units. When the loss is moderate to severe, these methods can clearly identify a loss of motor units, for example, as in a reduced interference pattern or a single motor unit firing pattern. EMG density measures of the number of motor units are further beset by the problem of relying on patient effort for obtaining activation.

Clinical EMG judgments about the number of motor units compare the rate of firing of single units with the total number of motor units. The determination of the rate of firing is one of the more difficult steps to make in standard EMG because of difficulty in obtaining sufficient patient control of motor units.
to isolate one or two units. When it is possible, the rate of firing of the motor unit initially activated is measured at the time the second unit begins to fire. Although recruitment analysis is reasonably reproducible and clinically reliable, it is usually a subjective judgment made by electromyographers on the basis of experience. Automated methods for formally quantitating the recruitment pattern have been developed. Attempts at this form of quantitation with decomposition of the EMG using the techniques developed by Dorfman and coworkers and by Guiheneuc are providing additional automation and quantitation of these measures. Further studies and technical developments may eventually allow recruitment analysis to provide more accurate estimates of the number of motor units in a muscle.

The amplitudes of compound muscle action potentials obtained with nerve conduction studies are directly related to the number and size of muscle fibers in a muscle group and indirectly to the number of motor units in the muscle group. The amplitude of a compound muscle action potential is a rough estimate of the number of motor units if a disease is known to be neurogenic and acute. Its value is limited by two factors. First, the amplitude is decreased in myopathies with loss of muscle fiber tissue. Second, the loss of muscle fiber activation that occurs if there is destruction of axons can be partially or fully compensated for by reinnervation from collateral sprouting of intact axons. Therefore, the amplitude of the compound muscle action potential cannot be used to obtain a reliable MUNE.

**BASIC ASSUMPTIONS OF QUANTITATIVE MUNE**

Quantitative MUNE can be obtained with needle EMG and motor nerve stimulation methods. Both the needle EMG and motor nerve stimulation approaches to MUNE make basic assumptions about the electrical characteristics of motor unit potentials that are described in this section. Four methods of making quantitative MUNE have been developed. Three of them use nerve stimulation and recording of compound muscle action potentials, and the fourth one relies on needle EMG. Variations of each of these four basic techniques continue to evolve with attempts at improving the accuracy and reliability of MUNE. Quantitative methods of MUNE were developed to improve on the accuracy and reliability of the subjective approaches described above. The newly developed techniques are based on either intramuscular needle EMG recordings or surface recordings of compound muscle action potentials of motor axon activation. Both of these techniques rely on several basic assumptions that must be understood to fully appreciate the advantages and drawbacks of quantitative MUNE.

Each method measures both the average size of the potentials generated by single motor units—single motor unit potentials—and the size of the compound muscle action potential obtained with supramaximal stimulation of a motor nerve. The MUNE is then obtained by dividing the supramaximal compound muscle action potential by the size of the single motor unit potential. The techniques differ in how the average size of the single motor unit potentials is obtained. The underlying assumptions about the measurement of the supramaximal compound muscle action potential and the measurement of the average single motor unit potential need to be understood to apply MUNE clinically.

Supramaximal stimulation of any peripheral motor nerve activates all the muscles innervated by that nerve distal to the point of stimulation. Therefore, measurements of the compound muscle action potential are the summation of activity from multiple muscles. For example, the median/thenar compound muscle action potential is the summation of the activity of the opponens pollicis, abductor pollicis brevis, flexor pollicis brevis, and, to a lesser extent, the lumbrical muscles. The ulnar/hypothenar compound muscle action potential is the summation of all the other intrinsic muscles of the hand. Thus, MUNE is more accurately an estimate of the number of motor units in groups of muscles rather than in a single muscle. Also, although the assumption that all motor axons are activated by supramaximal stimulation is generally true, it may not be the case in the presence of disease in which there are high-threshold axons (as in severe demyelination and regenerated axons). In these situations, the supramaximal compound muscle action potential may be difficult to obtain.

Whereas the methods of measuring the average single motor unit potential differ, they have common assumptions that need review. The most critical issue in determining the size of individual motor unit potentials is the adequacy of sampling of the entire population of single motor unit potentials in the muscle. In patients with severe neurogenic disease, it is possible to identify each motor unit and to obtain a reliable, reproducible, direct count of the number of motor units up to 10. This is the practical maximum of such direct counts. With more than 10 motor units, none of the methods allow reliable measures of each motor unit, so it is uncertain whether a true count has been made. In these cases, it is necessary to select a subset of the total population of motor units and then measure their size and calculate an average size for that subgroup. If all the motor units are nearly identical in size, then sampling a subset of motor units to obtain an average size of the single motor unit potentials is a valid approach. With greater variation in the size of single motor unit potentials, particularly if the range of sizes is not a normal Gaussian distribution, estimates of the true size become less reliable. Each of the methods of measuring the size of single motor unit potentials must address the bias in the selection of the single motor unit potentials that are measured and the adequacy of the sampling to determine whether a reasonable repre-
sentation of the total population of motor unit sizes has been obtained. The decrease in accuracy because of variation of the size of single motor unit potentials occurs particularly in severe chronic neurogenic processes. However, as the severity of damage of a neurogenic process increases and the variation in the size of single motor unit potentials increases, the ease of measuring these potentials also increases, thereby reducing the potential error from selection bias.

**MUNE METHODS**

A number of distinct methods for obtaining MUNE have been reported, each with its own advantages and disadvantages. Spike triggered averaging (STA), multiple point stimulation (MPS) and a statistical (STAT) method are most widely used. This demonstration will focus on STA and STAT. The others are summarized at the end of this handout.

**Spike-Triggered Motor Unit Potential Averaging**

Spike-triggered averaging relies on the ability to isolate single motor unit potentials by voluntary activation on needle EMG on a two-channel EMG machine. In this method, intramuscular motor unit potentials are measured with any one of several electrodes, including single-fiber EMG, bipolar concentric, standard concentric, or fine-wire electrodes. In each of these recording methods, individual motor unit potentials are isolated on the first channel, usually by an amplitude trigger window that selects potentials on the basis of peak amplitudes. Other criteria can also be used to select motor unit potentials. The accuracy of the method depends on the ability of the patient and electromyographer to activate, identify, and trigger individual motor unit potentials for a period long enough to allow the size of single motor unit potentials to be measured.

In spike-triggered averaging methods, the size of single motor unit potentials is measured on a second channel of the EMG machine that is triggered by the needle-recorded motor unit potential on the first channel. The activity averaged on the second channel from the surface electrode gives the size of the single motor unit potential. The technique requires the isolation of at least 10 and preferably 20 single motor unit potentials whose spike-triggered average can be recorded on the surface. The amplitude or area of the surface-recorded potentials is then used to calculate the average size of the single motor unit potentials in the muscle.

The same surface recording electrodes are used to record the supramaximal compound muscle action potential evoked with stimulation of the motor nerve to the muscle. MUNE is then determined by dividing the size (area or amplitude) of the supramaximal compound muscle action potential by the average size of the single motor unit potentials. A number of assumptions made in using this technique are possible sources of error. One, the method assumes that all motor unit potentials can be recorded at the surface. Studies by Brown and coworkers suggest that this is true in superficial muscles. Two, it assumes that voluntary activation recruits the full range of sizes of motor units. It is likely that this is not the case and that larger motor units are not activated with standard voluntary contraction. Despite these issues, the values obtained with the method are comparable to those expected on the basis of animal studies and those obtained with other methods of recording. de Koning and colleagues have modified the technique to provide a better representation by using macro-EMG needles to record the compound muscle action potential. It is assumed that a macroneedle provides a better representation of the full range of motor units, particularly those deeper in the muscle. Milner-Brown and Brown used the technique of microstimulation of nerve terminals in the endplate region to activate motor units recorded with a needle electrode. This reduces the bias in the selection of motor unit sizes that occurs with voluntary activation. Each of these methods gives comparable MUNE.

The spike-triggered averaging methods are generally more time-consuming and more complex to perform because of the need for two channels of recording: a motor unit potential triggering channel and a single motor unit potential averaging channel.

**Statistical Measurements**

Statistical MUNE uses direct stimulation of the motor nerve, similar to the all-or-none incremental and MPS methods (see below), but is conceptually different. With the statistical method, no attempt is made to identify the potentials associated with individual motor units. The method relies on the known relationship between the variance of multiple measures of step functions and the size of the individual steps when the steps have a Poisson distribution. Poisson statistics are used to calculate the number of quanta released from a nerve terminal at the neuromuscular junction when the individual quanta are too small to be distinguished, as in myasthenia gravis. Because the statistical method looks only at variance of the compound muscle action potential and does not require identification of individual components, it can be used when the sizes of single motor unit potentials are too small to be isolated, which is often the case in normal muscles and myopathies. It can also be used with high-amplitude compound muscle action potentials that require gains at which the single motor unit potential cannot be isolated. In pure Poisson statistics, the sizes of a series of measurements are multiples of the size of a single component. As shown in, where steps can be seen, a Poisson distribution has discrete values at which responses are found. A pure Poisson distribution has decreasing numbers at higher values. In this distribution, the variance of that series of measurements is equal to...
the size of the individual components making up each measurement and can provide an estimate of the average size of the single motor unit potential.

Similar to the other methods of estimating the size of the single motor unit potential, the statistical method assumes that each motor unit has a similar size and that it is the same size each time it is activated. Defects of neuromuscular transmission that result in varying sizes of the single motor unit potential cause inaccuracies in this measurement. With a larger number of components making up the summed potential, the distributions typically shift from a Poisson to a normal distribution. This produces an error of up to 10% in the MUNE.

In the statistical method, recording electrodes are applied as for standard nerve conduction studies, with the stimulating electrode taped firmly in place over the appropriate nerve. A “scan” of 30 stimuli of increasing stimulus intensity from threshold is applied to look for large increments in CMAP size that might represent single large SMUP. Four sets of stimuli are then applied in regions of suspected large SMUP. A sequence of 30 or more submaximal stimuli is given. The inherent variability of the threshold of individual axons causes their intermittent firing and all-or-none variations in the size of the compound muscle action potential. The variance of these 30 responses is calculated to obtain an estimate of the SMUP size. The occurrence of alternation with changing units that are activated does not modify the accuracy of the statistical method. Because the method is a statistical measurement, a somewhat different result is obtained each time. Therefore, multiple trials are needed to obtain the most accurate measurement. Experimental testing with trials of more than 300 stimuli has shown that repeated measurement of groups of 30 until the standard deviation of the repeated trials is less than 10% provides a close estimate of the number obtained with many more stimuli.

All-or-None Increments in the Compound Muscle Action Potential

All-or-none increment measurement, introduced by McComas, was the first method used for quantitative MUNE. The method is deceptively simple and provides the easiest and most direct and reliable method of obtaining MUNE. It is based on the well-known all-or-none characteristic of the activation of peripheral motor axons with electrical stimulation. In the incremental method, the stimulus current is finely controlled in very small steps designed to allow isolated stimulation of individual motor units in a progressive fashion. For example, if a muscle contains only two motor units, the compound muscle action potential consists of the single motor unit potentials of these two potentials only. Incremental testing with slowly and gradually increasing current will show no responses with stimuli below the threshold of the axons of both motor units. When the threshold of one of the axons is reached, the axon is fully activated and the compound muscle action potential suddenly changes from no response to the response of the single motor unit potential. This single motor unit potential is in the range of 50% of the total area. When the threshold of the second axon is reached, this axon also fires and the supramaximal compound muscle action potential is obtained. Moving the stimulus current up and down across these thresholds produces stepwise activation of two steps, that is, the first single motor unit potential and then the full compound muscle action potential. If there are three motor units in the muscle, three steps would be recorded, and similarly for larger numbers of motor units. In this technique, the size of the single motor unit potential is estimated from the incremental change in the compound muscle action potential, with control of the stimulus current and progressively increasing numbers of motor units. The more of these distinct steps of the total compound muscle action potential that can be measured, the more reliable the MUNE becomes with incremental measurements.

If the stimulation selectively activates larger or smaller motor units, there could be a selection bias in the single motor unit potentials used to determine the average size. Also, the occurrence of alternation may result in another error in the measurement.

Alternation is best illustrated by an example of a muscle containing three motor unit potentials of slightly differing size but of nearly the same threshold. In this situation, there could be as many as seven different size steps. Because of small variations in threshold of individual axons, an axon that is activated first in one trial may be activated second or third in subsequent trials. Thus, the sizes of potentials that could be obtained when there are three motor units of different sizes, A, B, and C, are those generated by A alone, B alone, C alone, by A and C, B and C, A and B, and by A, B, and C together. Thus, three single motor unit potentials might be recorded as three to seven steps.

Several modifications have been developed to minimize these errors: (1) the use of automated computer measurement of the templates of different single motor unit potentials to reduce the likelihood of measuring alternation, (2) stimulation at different points along the nerve (multipoint stimulation) to isolate only single motor unit potentials, (3) the use of recording electrodes of different sizes and shapes, (4) the use of an automated method of incrementing the stimulus size, and (5) microstimulation of single nerve terminals at the endplate region.

The McComas incremental compound muscle action potential technique uses whatever average values are obtained for the size (amplitude or area) of the single motor unit potential and compares them with the supramaximal size of the compound muscle action potential. Normal value determinations by several authors have shown that the mean normal MUNE for median/thenar muscles is about 350 (range, 100 to 500).
other well-studied muscle, the peroneal/extensor digitorum brevis, has been estimated to have 200 motor units (range, 50 to 300).

The incremental technique becomes unreliable when the motor unit potentials become quite small, as in severe myopathies or with nascent motor unit potentials. The inability to record the steps results in underestimation of MUNE.

The method of incremental compound muscle action potential is direct enough that it should be learned by every electromyographer. It can be applied to patients in whom loss of motor units allows the remaining axons to be stimulated selectively and recorded as incremental steps by stimulus control.

**F-WAVE MEASUREMENTS**

F waves have been suggested as a method for determining the size of the single motor unit potential. Supramaximal activation of all the motor axons in a peripheral nerve is associated with antidromic activation of some of the anterior horn cells. The small proportion of anterior horn cells activated antidromically produces small late potentials, F waves. Repeated supramaximal stimuli activate different anterior horn cells and produce different F waves. Recording a range of sizes of F waves can be used to estimate the average size of the single motor unit potential. This average size can then be divided into the supramaximal compound muscle action potential to obtain MUNE. Brown and colleagues have shown that the drawbacks of this method are the alternation described above for the increment method and the common activation of multiple rather than single motor units. Both of these drawbacks result in overestimating the average size of the single motor unit potential and in underestimating the MUNE. Automated correction of these drawbacks by submaximal stimulation and template matching may make the method clinically useful.

**REFERENCES**
