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ABSTRACT: The statistical method of motor unit number estimation (MUNE) uses the natural stochastic variation in a muscle's compound response to electrical stimulation to obtain an estimate of the number of recruitable motor units. The current method assumes that this variation follows a Poisson distribution. We present an alternative that instead assumes a binomial distribution. Results of computer simulations and of a pilot study on 19 healthy subjects showed that the binomial MUNE values are considerably higher than those of the Poisson method, and in better agreement with the results of other MUNE techniques. In addition, simulation results predict that the performance in patients with severe motor unit loss will be better for the binomial than Poisson method. The adapted method remains closer to physiology, because it can accommodate the increase in activation probability that results from rising stimulus intensity. It does not need recording windows as used with the Poisson method, and is therefore less user-dependent and more objective and quicker in its operation. For these reasons, we believe that the proposed modifications may lead to significant improvements in the statistical MUNE technique.

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STATISTICAL MOTOR UNIT NUMBER ESTIMATION ASSUMING A BINOMIAL DISTRIBUTION

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Many neurogenic disorders are characterized by a reduction in the number of recruitable motor units (MUs) in affected muscles. A reliable count or estimate of this number is then of obvious importance, both for diagnostic purposes and for following disease progression or therapeutic effects. Comprehensive overviews of the available electrophysiological motor unit number estimation (MUNE) techniques, their underlying assumptions, and advantages and disadvantages have been provided elsewhere.^{4,11} All of these MUNE methods start by trying to find a mean MU potential (MUP) that is representative of the muscle as a whole. The MUNE is subsequently determined by dividing this representative MUP into

the maximal compound muscle action potential (CMAP), which is generated by the muscle after supramaximal electrical stimulation of its motor nerve.

The various methods differ primarily in their means of obtaining a representative sample of MUPs, as well as in how they deal with the probabilistic activation of motor units. When stimulus intensity is increased, starting from the recruitment threshold of an individual MU, the firing probability of this MU changes gradually from 0 (never activated) to 1 (always activated). When the recruitment ranges (range of stimulus intensities over which the firing probability increases from 0 to 1) of a number of MUs overlap, any combination of these units can be activated upon successive stimuli with equal strength, a phenomenon known as alternation. The statistical method of MUNE uses the stochastic properties of the resulting variation in the recorded CMAP amplitude to obtain an estimate of the mean electrical size of the MUs.^{2,8}

The statistical method is implemented as proprietary software on one of the commercially available

Abbreviations: CMAP, compound muscle action potential; EMG, electromyography; MU, motor unit; MUNE, motor unit number estimation/estimate; MUP, motor unit potential; SI, stimulus intensity

Key words: alternation; binomial; motor unit number estimation; Poisson; statistical method

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electromyography (EMG) systems (Viking product line, Nicolet Biomedical, Madison, WI). It is easy to perform in clinical practice, fairly sensitive to changes in motor unit number, and yields reproducible results.^{5-10,12} For these reasons, it has become one of the most commonly used MUNE techniques. The statistical technique has some limitations, however. As discussed recently by Lomen-Hoerth and Slawnych,⁸ its validity depends on the degree by which several assumptions are met. The most important of these is the assumption that the variation in CMAP size follows a Poisson distribution. In turn, this Poisson distribution assumption requires that all MUs undergoing probabilistic activation at a particular stimulus intensity respond independently to each stimulus pulse, have MUPs of the same size, and have the same probability (p) of activation. Moreover, and this defines the distribution as Poisson rather than more generally binomial, p should be small (i.e., much closer to 0 than to 1).

In this study, we argue that the latter assumption is not met in statistical MUNE recordings. In a vast majority of cases, p is larger than 0.05 (a value that may be taken as maximum for a Poisson distribution to be a valid description). In fact, in many cases, p approaches 0.5. The resulting distribution then is binomial rather than Poisson. We show how the assumption of a binomial distribution alters the existing statistical MUNE technique, both in theory and in its practical application. We first present a mathematical description of the Poisson method and the binomial method. This theoretical section is followed by an analysis of the performance of the two methods, using computer simulations and the results of a study of normal subjects.

THEORETICAL BACKGROUND

The Poisson Method. The mathematical basis of the Poisson distribution technique has recently been described by Lomen-Hoerth and Slawnych.⁸ To allow a comparison with the binomial distribution, we present it in a slightly different form.

Basically, the Poisson statistical MUNE method distinguishes MUs into three groups: MUs that are always active ($p = p_s = 1$); MUs that are probabilistically activated (with identical, supposedly small p_s value; $p_s \approx 0$); and MUs that are never activated ($p_s = 0$) at a particular stimulus intensity (s). Only the observed CMAP amplitudes or areas of the second group of MUs ($0 < p_s < 1$) are analyzed. The method assumes that the MU potential sizes of these MUs are identical, implying that the CMAP size $A_s(i)$ varies in integer multiples $I_s(i)$ of the (unknown)

single motor unit size ΔA with stimulus number (i). The distribution of these multiples, that is, of the values $I_s(i) = [A_s(i) - \min(A_s)]/\Delta A$, is assumed to be Poisson. In this expression for I_s , $\min(A_s)$ is the smallest observed CMAP size at the particular stimulus intensity, s . It is assumed to be the total response of the first group of always active MUs (with $p = 1$) and represents a baseline shift in the response sizes used for the analysis.

Characteristic for a Poisson distribution is the equality of its mean and variance: $\text{mean}(I_s) = \text{var}(I_s)$. From the above expression for I_s it follows that:

$$\text{mean}(I_s) = [\text{mean}(A_s) - \min(A_s)]/\Delta A \quad (1a)$$

$$\text{var}(I_s) = \text{var}(A_s)/\Delta A^2 \quad (1b)$$

Hence, the mean MUP size ΔA can be estimated from:

$$\Delta A = \text{var}(A_s)/[\text{mean}(A_s) - \min(A_s)], \quad (2)$$

with $\text{var}(A_s)$ the variance of the observed CMAP sizes. The Poisson MUNE N_p easily follows as:

$$N_p = \text{CMAP}_{\max}/\Delta A \quad (3a)$$

or:

$$N_p = \text{CMAP}_{\max} * [\text{mean}(A_s) - \min(A_s)]/\text{var}(A_s) \quad (3b)$$

with $\text{CMAP}_{\max}$ the response of the muscle to supra-maximal stimulation.

Problem Statement. The Poisson method of MUNE assumes that the group of probabilistically active MUs is fairly small. The minimum of the observed CMAP sizes recorded in response to a series of constant-intensity, submaximal pulses is taken to represent the case where all MUs in this group with $0 < p_s < 1$ are not recruited. This assumption is questionable, as demonstrated in the Appendix, but it will be adopted here.

For healthy subjects, the observed range of CMAP sizes is of the order of 10% of the maximum CMAP.^{5,9} The variably active group of MUs may therefore be assumed to constitute about 10% of the total population; for instance, $n_s = 25$ MUs out of a population of $N = 250$. For the sake of simplicity, we will assume them to be of unitary size. This implies that from this population 26 different values can be observed: from 0 when no MU is active to 25 when

they all are. The probability with which each of these observable values occurs is determined by the combined probabilities of activation of the 25 MUs. To obtain a Poisson distribution, this probability has to be equal for all MUs and small (e.g., 0.05). The resulting probability density function is shown in Figure 1. It has a low mean, $\mu_s = n_s p_s = 25 \cdot 0.05 = 1.25$, and is strongly skewed to the right. At this point, a paradox arises, as the range of amplitudes in this example is closer to 0–5 than to the 0–25 that was the premise of the example. This paradox points to $p_s > 0.05$ or to a number of MUs in the probabilistically active group of more than 25.

Figure 2 shows representative results of four series of CMAP recordings at different stimulus intensities in a healthy subject (for a description of the method of acquisition, see Methods). The histograms on the right show the distribution of the observed values. The symmetry of the histograms is apparent. There is no skew of significance, implying that the mean of the distribution is close to the center of the range of observable values. Because this mean is determined fully by the two parameters n and p , the disparity between the means in Figures 1 and 2 (relative to the minimum and maximum value of the distributions) again indicates that either the group of variably active MUs is larger, or the Poisson distribution (small- p) assumption is not met, or both.

It is shown in the Appendix that assuming a larger group of probabilistically active MUs cannot solve the inconsistency between Figures 1 and 2. By contrast, abandoning the assumption that the prob-

ability of activation, p_s , of the n_s MUs is small, and allowing it to vary between 0 and 1, implies that the distribution mean can take any value between 0 and n_s for n_s MUs of unitary size. However, the Poisson distribution is no more or less than the small- p member of the wider class of binomial distributions (with arbitrary p). Lifting the small- p assumption is, therefore, identical to assuming a binomial instead of a Poisson distribution. In other words, when a group of n_s probabilistically active MUs with equal-sized MUPs generates a distribution with a high mean (i.e., a mean that is larger than a few percent of the range of potentially occurring values), then the observed distribution is binomial rather than Poisson.

The Poisson MUNE technique usually employs so-called windows to limit the range of response sizes included in the analysis.^{5,7,9} These windows are placed relative to the distribution mean. Most often, the stimulation intensity is adjusted until the distribution mean falls at either the lower quarter of the window (at 25% of the window width) or at its center (50% of the window width). In terms of the activation probability p , these positions must imply p -values of 0.25 and 0.5, respectively. Therefore, in our opinion, the Poisson MUNE method uses Poisson calculation algorithms for a distribution that by all descriptions is binomial and not Poisson. Of course, treating it mathematically as if it were Poisson is possible (mainly a matter of calculating mean and variance of the distribution), but is bound to generate erroneous MUNE values.

The Binomial Method. As for the Poisson distribution, the binomial method that we propose also requires the assumption that all MUs that are variably active have the same size. In contrast to the Poisson method, however, the binomial method does not distinguish MUs into three groups, but assumes that they all have the same activation probability. (This choice may seem surprising given the physiological reality in which the groups exist. It was motivated by our hypothesis that at most stimulus intensities the second, probabilistically activated group of MUs is, in fact, large compared to the other groups. This issue is discussed extensively in the Appendix.) Let the electrical size of the MUs again be ΔA and their probability of firing at a given stimulus intensity (s) be p_s . If there are N motor units, then the expected number of responding MUs is $\mu_s = N p_s$ and the expected CMAP size equals $\mu_s \Delta A$. This expected CMAP size can be estimated by the mean of the observed values of the CMAPs: $\text{mean}(A_s) \approx \mu_s \Delta A$. When it is noted that the observed distribution mean can also be expressed as:

FIGURE 1. Probability density function for a Poisson distribution of CMAP sizes, generated by an assumed population of 25 MUs of equal size. Each of these MUs is activated with the same probability, $p = 0.05$, so the mean of the corresponding distribution is 1.25.

FIGURE 2. (Left) Representative results from a healthy subject, showing CMAP sizes as percentage of the maximum CMAP in response to a series of 180 stimuli at four different stimulus intensities (different symbols). **(Right)** Histograms illustrating the distribution of CMAP sizes for each of the four intensities.

$$\text{mean}(A_s) \approx \mu_s \Delta A = N p_s \Delta A = p_s N \Delta A = p_s \text{CMAP}_{\max} \quad (4)$$

it is clear that p_s , the activation probability of the MUs at a particular stimulus intensity, s , can be obtained easily from the data as:

$$p_s \approx \text{mean}(A_s) / \text{CMAP}_{\max} \quad (5)$$

From first principles, if the distribution I_s of the multiples of the MUP size ΔA (i.e., the underlying process) is binomial, then its variance is given by:

$$\text{var}(I_s) = \sigma(I_s)^2 = \sigma_s^2 = N p_s [1 - p_s] \quad (6)$$

with σ denoting standard deviation. In that case, the variance, $\text{var}(A_s)$, of the observed CMAP size distribution is $\sigma_s^2 \Delta A^2$, which can also be estimated from the data. As can be verified by substitution of $\text{mean}(A_s) \approx N p_s \Delta A$ and $\text{var}(A_s) \approx N p_s [1 - p_s] \Delta A^2$, it follows that the binomial MUNE, N_b , of the true MU number, N , can be deduced from:

$$\text{var}(A_s) / \text{mean}(A_s)^2 \approx (1 - p_s) / N p_s \quad (7a)$$

or:

$$N_b = [(1 - p_s) / p_s] * \text{mean}(A_s)^2 / \text{var}(A_s) \quad (7b)$$

Finally, inserting Equation (5) into the above expression for N_b yields:

$$N_b = [\text{CMAP}_{\max} - \text{mean}(A_s)] * \text{mean}(A_s) / \text{var}(A_s) \quad (8)$$

This method is different from all existing MUNE methods in that it does not need a representative MUP to be divided into the maximum CMAP. The motor unit size, ΔA , is estimated simultaneously with the motor unit number, N_b , from the distribution characteristics. When desired, ΔA can be obtained by dividing the maximum CMAP by the MUNE.

A concluding remark concerns the relation between the Poisson and binomial distributions. From Equation (6) for binomial variance, it can be seen that, when p_s is small compared to 1 (so that $1 - p_s$ remains close to 1), then:

$$\sigma_s^2 = N p_s [1 - p_s] \approx N p_s = \mu_s \quad (9)$$

This equality of mean and variance is characteristic for the Poisson distribution and illustrates that the Poisson distribution is the small- p limiting case of the binomial distribution. Equation (9) also shows that the factor of $(1 - p_s)$ functions as a moderator, allowing adaptation of the distribution shape to an increase in activation probability (Fig. 3A).

Thus, from the responses to a series of submaximal stimuli, the average CMAP size is measured together with its variability, expressed as variance. Together with the recorded maximal CMAP, these two variables suffice to estimate the size of the underlying population (the number of motor units). The proposed method differs from the existing Poisson method in the distribution that is chosen to explain the observed variance (binomial rather than Poisson). This choice appears to be critical and largely determines the outcome, as shown in what follows.

METHODS

Simulation Study. To simulate alternation, a computer model was constructed and implemented in Matlab (The MathWorks, Natick, MA). For this purpose, we started from the observation that the CMAP size as a function of stimulus strength (the scan in

the statistical MUNE technique) usually has a sigmoid shape. Previous work by Doherty and Brown suggests that MU activation depends more on relative proximity of the nerve fibers to the stimulus electrodes than on fiber diameter or MU size.³ This implies that the steep increase in CMAP size in the center of the scan is not caused by an increase in the size of the newly recruited MUs, but rather by an increase in the number of MUs with similar recruitment thresholds. For a pool of 250 motor units, recruitment thresholds were therefore drawn at random from a normal distribution (see histogram in Fig. 4A). For this purpose, recruitment threshold was defined as the stimulus intensity at which the MU was active in 50% of the cases. The mean of the normal distribution corresponds to the stimulus intensity (SI) at which the scan shows the steepest increase in CMAP size.

The firing probability of a particular MU was assumed to increase linearly from 0 to 1 with SI, over a range that was centered around the recruitment threshold of that MU (Fig. 4A; solid line for an MU with recruitment threshold at 27). This range, which runs from an SI of 12–41 for the MU shown, will henceforth be referred to as the recruitment range. The rate of increase in firing probability (i.e., the slope of the linear increase and, hence, the width of the recruitment range) was assumed to be the same for all MUs, and all MUs had the same (unitary) size. This implies that, in the model, MUs differed only by their recruitment threshold, as represented by the histogram in Figure 4A. However crude this approximation may seem, it results in a CMAP curve (or scan; see Fig. 4B) that is similar to physiological scans. As will be discussed in the Appendix, we believe that this model follows the physiology of MU recruitment more reliably than the assumptions behind either the Poisson or the binomial estimation approach. In that sense, it is a powerful tool for comparing both MUNE methods, showing an additional advantage over experimental studies in that the true number of MUs is known (i.e., a gold standard is available).

For the pool of 250 motor units, the CMAP size distribution was simulated for a range of SIs, from 0 to supramaximal. At each intensity, 1000 stimuli were applied. Depending on the firing probability of a particular MU at that SI, it was determined by chance whether it was or was not activated in response to a particular stimulus. If activated, it added a response size 1 to the CMAP size. Hence, at supramaximal stimulus intensities, CMAP size is 250. A summary of the model output (CMAP size) is shown in Figure 4B. The binomial MUNE and Poisson

FIGURE 3. (A) Examples of binomial distributions, for varying probability of activation (columns) and generated by a varying population size (top row: 25 MUs; middle row: 250 MUs; bottom row: 2500 MUs). The binomial distribution is widest when $p = 0.5$, becoming thinner at higher or lower activation probabilities (compare columns). Furthermore, its relative width (width expressed as percentage of the maximal observable value or maximum CMAP) decreases with an increase in the size of the contributing population (compare rows). Note the similarity between the top left figure and Figure 1, illustrating that the Poisson distribution shown in Figure 1 is the small- p member of the family of binomial distributions. **(B)** Distributions obtained with the computer model of alternation (see Fig. 4 and text). The correspondence between these curves and those in the middle row of **(A)** indicates that the CMAP size distributions generated by the model can be considered binomial in nature.

FIGURE 4. (A) Model input. The histogram shows the (normal) distribution of the recruitment thresholds of 250 MUs. Recruitment threshold was defined as the stimulus intensity at which the MU was active in 50% of the cases. Stimulus intensity is in arbitrary units (a.u.). The line shows the recruitment range for one of the MUs (from 12 to 41 a.u., with threshold at 27). **(B)** Summary of model output, showing mean (black solid line), maximum, and minimum (gray solid lines) of the resulting CMAP size distribution as a function of stimulus intensity. The width of the distribution (dashed line) is the difference between the maximum and minimum.

MUNE were calculated for this simulated CMAP size distribution using the mathematical approach in the previous section. To assess the influence of recording windows, calculations were performed for three situations: 5% and 10% centered windows, and a 10% window with the distribution mean in its lower quarter instead of center. In these cases, the window width is expressed as percentage of the maximum CMAP and its position is expressed relative to the distribution mean at a specific stimulus intensity. The horizontal gray bars in Figure 4B illustrate the use of a 5% and 10% window.

To assess the effect of motor unit loss on the MUNE, simulations were repeated for a model input number of MUs ranging from 10 to 350. In each case, results for four stimulus intensities were simulated. The lowest yielded a mean CMAP size of 15% of the maximum CMAP, followed by 30%, 45%, and 60% means, in accord with the recommendations of Lomen-Hoerth and Olney.⁷ In this study, 10% centered windows were used for the Poisson calculations.

Experimental Study. The motor unit number was estimated in the thenar muscle group of 19 healthy subjects (8 males, 11 females) without neuromuscular complaints. Their mean age was 42.7 years (range 22–63 years). The experimental protocol was approved by the medical ethics committee of the Erasmus Medical Center. All subjects gave informed consent.

The statistical MUNE technique was performed using the MUNE500 program⁵ implemented on a Viking Select EMG machine (Nicolet Biomedical, Madison, WI). To record the potential at the skin

surface, we used 2.5-cm $\times$ 3.5-cm disposable, self-adhesive electrodes. The active electrode was placed over the belly of the abductor pollicis brevis muscle, and the passive electrode in the middle of the proximal phalanx of the thumb. The ground electrode was placed at the base of digits 3 and 4. The median nerve was stimulated percutaneously at the wrist, 8 cm from the active electrode. After determining the optimal position for the stimulating electrodes, these were taped securely to the wrist. Subjects were asked to recline and relax without speaking, with the hand in a pronated position at their side. As observed by Miller et al.,⁹ this approach to reducing baseline fluctuations and artifacts proved superior to restraining the hand or arm.

Recordings were started with the recording of the maximal CMAP, and a scan of the muscle's response to increasing stimulus intensity was made. Subsequently, four runs of tests were performed at stimulus intensities that evoked responses around 10%, 30%, 50%, and 70% of the maximum CMAP (slightly different intensities were used in the experiments compared to the simulations to ascertain a wide range of tested MUs). Each run consisted of 180 stimuli; stimulus frequency was 2 Hz.

For each subject, both the Poisson MUNE and the binomial MUNE were obtained from the recorded data. In both cases, the areas of the recorded CMAPs were used for analysis. The Poisson MUNE was calculated using only the data within a 10% centered window. Data were first assessed visually for the presence of motion artifacts, drifts of more than a few percent of the maximum CMAP, or spread.⁵ If any of these were present in a run, this run was

FIGURE 5. (A) Results of the Poisson (black) and binomial (top black-grey line) MUNE calculations for the simulated data. For the Poisson calculations, three types of recording window were used: 10% centered (solid); 10% low quarter (dashed); and 5% centered (dotted). In the first and third case, the window was centered around the distribution mean at a particular stimulus intensity. In the second, the window was placed such that the distribution mean fell at 25% of the window width. The x-axis shows the distribution mean as percentage of the maximal CMAP, and may be taken to represent stimulus intensity: 0 implies an SI that is just below the activation threshold of the lowest threshold motor unit, whereas 100 corresponds to a supramaximal SI and 50 to an SI that on average activates 50% of the MUs. **(B)** Percentage of responses that fell outside of the recording windows.

excluded from further analysis. Next, as in the conventional Poisson method, data were subdivided into series of 30 consecutive responses for which binomial and Poisson MUNE values were determined. The MUNE of a particular run was taken to be the median of the resulting six MUNE values. This procedure reduces the effect of a slight drift in the recorded CMAP sizes compared to analyzing all 180 responses together. We present MUNE results as median values rather than means because the results were not normally distributed. For the same reason, the median of MUNE values that were obtained for the four runs was taken to obtain an overall estimate. An overall MUNE was determined only if at least three runs in a particular subject were artifact-free.

RESULTS

Figure 5 shows the results of the simulation study. Figure 5A demonstrates that the binomial estimate is nearly independent of SI, with a mean of 276 ± 3 , and 5th and 95th percentiles of 254 ± 1 and 299 ± 1 , respectively (errors represent standard error of the mean, evaluated over 10 simulation runs). This implies that for the simulated data, the binomial method overestimates the correct outcome of 250 by approximately 10%. The Poisson estimate, by contrast, strongly depends on SI. For low intensities ($SI < 5$), the Poisson values are nearly as high as the binomial MUNE values, whereas in the middle range they decrease to below 100. At high stimulus intensities ($SI > 95$), the Poisson MUNE returns to the initial values. The use of windows only slightly modulates

this behavior, as may be deduced from the three examples in Figure 5A. For all three window settings used (10% centered, 10% low quarter, and 5% centered), reasonably accurate results compared to the known model input of 250 MUs are obtained only for very low or very high stimulus intensities.

Figure 6 indicates that the binomial MUNE decreases proportionally with a decrease in number of MUs present, overestimating the true MUNE by approximately 10%. This factor appears to be independent of the number of motor units present. By contrast, the Poisson method decreases also linearly, but not proportionally, with a decreasing MU number over most of the tested range. This results in a more gradual decline in MUNE. Consequently, the Pois-

FIGURE 6. Model results for a range of motor unit numbers (model input). The gray line marks the ideal situation, in which the MUNE method would be able to determine the number of MUs with perfect accuracy. Results are shown for four stimulus intensities, resulting in distribution means at 15% (solid line), 30% (dashed line), 45% (dotted line), and 60% (dashed-dotted line) of the maximum CMAP size.

son MUNE at first is lower than the true MU number, but with increasing MU loss may eventually be higher (for numbers of MUs below approximately 50; left of Fig. 6B). The extent of over- or underestimation in the Poisson method is, therefore, highly variable.

The experimental MUNE results confirm the higher binomial values. For the Poisson method, a median MUNE value of 88 was obtained (range 65–137); for the binomial method, median MUNE was 303 (range 160–479). Both the binomial and the Poisson result agree well with the results of the simulation study. The experimental results are based on the data of 12 of the 19 subjects and a total of 37 runs. In the other 7 subjects, 11 runs yielded valid data, but in only one or two runs per subject. Data from 28 runs were excluded for various reasons: severe drift in 9 subjects (12 runs); spread in 4 subjects (4 runs); motion artifacts in 2 subjects (2 runs); and steps in 4 subjects (4 runs). In a few cases, extremely high values (>1000) could be observed. In these cases, the variance of the distribution was far too small to yield reasonable MUNE values. The results for these runs were also excluded from the analysis (6 runs in 4 subjects).

DISCUSSION

Simulation Study. The simulation model provides a simplified version of reality in that it assumes that MUs differ only by their recruitment threshold. Furthermore, we assumed these thresholds to be quite close together with respect to the width of the SI range over which the firing probability, p , of an individual MU increases from 0 to 1 (Fig. 4A). Hence, at intermediate stimulus intensities in particular, for a vast majority of MUs, this probability is larger than 0 and smaller than 1. Because the binomial method is based on the assumption that all MUs are probabilistically active, this agreement explains at least in part the good performance of the binomial MUNE method when applied to the simulation results (Figs. 5 and 6). In other words, the reliability of the model results presented here depends on the true size of the probabilistically active MU population at a given SI. As discussed in the Appendix, we have several reasons to believe that the variably active group is, indeed, large and that our model provides a sufficiently accurate description of the underlying physiology. Hence, its output can be used to assess the behavior of the MUNE with changes in the calculation method, stimulus intensity, window size, and window position.

When p is truly small, as is the case for low SIs, the Poisson estimate is as good as the binomial estimate (Fig. 5A), as expected on theoretical grounds. However, for most stimulus intensities, p cannot be that small. Consequently, the Poisson method severely underestimates the number of motor units. Changing window size or window position cannot adequately correct for this deficit. By contrast, the binomial method allows p to take any value between 0 and 1. In that way, it can accommodate the changes in the distribution characteristics that are the result of a rising stimulus intensity (Fig. 3). The resulting MUNE is much more accurate than the Poisson estimate.

Irrespective of the size of the variably active group of MUs or the calculation method used (Poisson or binomial), the statistical method of MUNE assumes that all MUs that are probabilistically active have the same activation probability, p . Only when this condition is met is the distribution truly binomial (or Poisson). In the physiological situation, however, the observed variation will be determined by the combination of all individual probabilities of activation, $p_s(i)$, of all motor units at a particular stimulus intensity, s , and the result generally will not exactly follow a binomial distribution. That the observed CMAP size distribution at a certain SI would be binomial remains, therefore, an approximation. Nevertheless, the very good agreement between the model results and the binomial probability density function as presented in Figure 3B and the middle row of Figure 3A, respectively, indicates that it is a sufficiently good approximation indeed.

Experimental Study. In its execution, the binomial test does not differ from the Poisson method (except for the selection of windows, which is superfluous), requiring but a minor change in the software algorithms that calculate the MUNE. However, as anticipated from the simulation study, the experimental MUNE values obtained with the binomial method (303; range 160–479) are much higher than those obtained with the Poisson method (88; range 65–137). Results for healthy subjects using MUNE techniques other than the statistical technique yield the same MUNE values that range from low values between 150 and 200 (mostly for the spike-triggered averaging technique) and high values up to 360 for the increment counting method.¹¹ The median binomial MUNE of 303 fits well into this range.

Our results for the Poisson method are in fairly good agreement with previous results for this method.^{6,7,9,12} In these studies, MUNE values between approximately 60 and 120 were found, with most

values around 100. Although most of these results have been obtained for the hypothenar muscles, recent evidence⁵ suggests that Poisson MUNE values obtained for the thenar and hypothenar muscles are similar, and hence that the aforementioned studies can be used to yield valid reference values for our study. In this comparison, it should also be recognized that, in determining the overall MUNE value of 88, we chose to use the median rather than the mean of the results of the four runs, because these results were not normally distributed. The mean was 102, which may be considered to be in even better agreement with previous results than the median value.

During the acquisition of the experimental data, we did not correct for drifts by slightly readjusting the stimulus intensity, as was done in some other studies.^{5,9} Because we do not understand the nature of the cause of these drifts, we did not want to risk altering the variably active pool of MUs deliberately. The consequence of this choice was a large number of runs (12 out of 76) that had to be excluded from further analysis. For similar reasons, we did not repeat recordings at a different SI if we found steps to be present, but preferred to adhere to the predetermined settings of 10%, 30%, 50%, and 70%. In a clinical setting, of course, different choices may be made, which will yield a higher number of useful runs.

In six runs, extremely large binomial MUNE values were obtained (MUNE >1000). In these cases, Poisson values were also large (range 127–519). Inspection of the corresponding distributions indicated that their width was very small. Because the width of a distribution is directly related to its standard deviation, and hence to its variance, it follows from Equations (3b) and (8) that MUNE values will be high. Most likely, in these cases only a relatively small number of the total population of MUs are variably active. Hence, the basic assumption of the binomial method is incorrect that all MUs are probabilistically active with equal probability. Future improvements in the binomial method should provide some measure to correct or at least warn for this situation. This problem has now been remedied by exclusion of runs yielding values above the somewhat arbitrary limit of 1000.

Finally, our simulation studies (Fig. 6) suggest that the binomial MUNE technique is more sensitive to a decrease in motor unit number than the Poisson method. Clinical studies are required to corroborate this. In this respect, it is important to note that the binomial MUNE method assumes a stable all-or-none response of motor axons following electrical

stimulation. In this sense, it does not differ from the Poisson method. Any CMAP variability introduced by unstable MUPs (such as in neurogenic conditions) cannot be separated from variability due to alternation and will affect the resulting MUNE.

APPENDIX: NUMBER OF SIMULTANEOUSLY ACTIVE MOTOR UNITS

Figure 1 shows the Poisson probability density function that follows from the assumption that only a small number of MUs (25 of a pool of 250) is probabilistically active with a small probability, p . It does not resemble most experimentally obtained distributions. Increasing the number of MUs will increase the absolute value of the mean of the Poisson distribution (assuming $p = 0.05$ and $n = 250$ will generate a Poisson distribution with mean $\mu = 12.5$; that is, a mean that is located in the middle of a 10% recording range with values from 0 to 25), but its value as percentage of the distributions' maximum observable value (250 instead of 25 in this example) remains the same. The long tail of the distribution, of values with a very small chance of being observed, is an integral part of a Poisson distribution and cannot be omitted. This is true even when this tail extends beyond the maximum observable value (maximum CMAP), leading to internal inconsistencies in the Poisson method. Because at higher stimulus intensities (SIs) this tail will extend further beyond the physiologically possible maximum, this internal inconsistency of the Poisson method becomes more pronounced with a rise in SI.

Furthermore, the aforementioned situation does not yet take into account the potential existence of a group of MUs that is never active at a particular SI. The size of this continuously inactive population cannot be estimated from the distribution, be it Poisson or binomial, because that would require registration of at least one occurrence of simultaneous activity of all probabilistically active MUs. As illustrated in Figures 1 and 3, the probability of such an occurrence in a finite series of recordings is nil for all except the highest stimulus intensities. More specifically, this combined activity of all probabilistically active MUs is not reflected by the highest observed value. Similarly, the smallest observed value in a series of recordings generally does not represent the situation in which none of the MUs is active either. Thus, from the information available in the distribution, it is impossible to determine the sizes of the always active, variably active, and never active groups of MUs. All that can be said is that the size of the variably active group is: (a) less than or equal to

that of the total population; and (b) larger than or equal to the percent width of the observed CMAP size distribution. Both extremes (the first assumed in our methodology, the second in the conventional method) yield erroneous MUNE values, because physiologically at least two groups will exist at most stimulus intensities. However, one of the approximations will reflect physiology more accurately and, hence, result in a more accurate MUNE.

Little is known about the number of MUs (as percentage of the total number) that are probabilistically active at a certain SI, that is, about the extent of alternation. Work by Brown and Milner-Brown¹ showed that, beyond the one or two lowest threshold MUs, recruitment ranges quickly started to overlap. Although they were able to record some single MU stimulus–response curves, they could not determine how closely together the thresholds were for all except the lowest stimulus intensities. Furthermore, they did not relate their findings to the stimulus–response curve for the muscle as a whole.

Because of this lack of direct evidence for either of the two assumptions, we had to revert to indirect evidence instead. First, such evidence was found in the behavior of distribution width with stimulus intensity. If a large proportion of the MUs are probabilistically active with a probability that increases with stimulus intensity, then the width of the distribution will increase until intermediate stimulus intensities are reached and then decrease (Fig. 3). This behavior of distribution width with increasing stimulus intensity was experimentally confirmed. Second, support for a large variably active population and, hence, for the current model parameter settings, follows also from the good quantitative agreement between experimental results and the simulations—for instance, in the resulting MUNE values. Third, from our clinical experience with single-fiber EMG elicited by surface stimulation over the motor nerve, we know that recruitment ranges of individual muscle fibers tend to be in the same order of magnitude as the recruitment range of the muscle. This correspondence between the behavior of individual muscle fibers and of the muscle as a whole suggests that the MUs have stimulus–response curves that are

very close together. Finally, we point out that a small distribution width percentage-wise is not necessarily related to a small group of probabilistically active MUs. It may equally well be the result of a large population of probabilistically active MUs (Fig. 3). Based on the aforementioned evidence, we chose to implement and evaluate a binomial MUNE method that is based on the assumption that all MUs are probabilistically active.

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