STATISTICAL ANALYSIS OF MOTOR UNIT FIRING PATTERNS IN A HUMAN SKELETAL MUSCLE

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ABSTRACT A statistical analysis of the firing pattern of single motor units in the human brachial biceps muscle is presented. Single motor unit spike trains are recorded and analyzed. The statistical treatment of these spike trains is as stochastic point processes, the theory of which is briefly discussed. Evidence is presented that motor unit spike trains may be modelled by a renewal process with an underlying gaussian probability density. Statistical independence of successive interspike intervals is shown using scatter diagrams; the hypothesis of a gaussian distribution is accepted at the 99th percentile confidence limit, chi-square test, in 90% of the units tested. A functional relationship between the mean and standard deviation is shown and discussed; its implications in obtaining sample size are presented in an appendix.

The results of higher order analysis in the form of autocorrelograms and grouped interval histograms are presented. Grouped interval histograms are discussed in the context of motor unit data, and used to confirm the hypothesis that a stable probability density function does not represent a good model of the data at this level of analysis.

INTRODUCTION

The smallest functional unit of a skeletal muscle is the motor unit, defined as a motor neuron and all the muscle fibers it innervates. A single action potential travelling down the axon excites the muscle fibers which respond, each with a propagated action potential and a mechanical twitch. Normally, contraction by the motor unit results from a rapid succession of action potentials and a simultaneous train of twitches which fuse to a greater or lesser extent to yield a steady pull. A considerable body of literature exists on properties of these spike trains, specifically the firing frequency range and the relation between frequency and the muscle output variables (length and tension).

Rosenfalck (1) has reported that interspike intervals of successive spikes are normally distributed. With this exception, studies on the statistical properties of spike trains are virtually nonexistent. Thus, no criteria exist to determine the inter-

FIGURE 1 Typical needle electrode records. A, Single unit; B, Two units firing at different frequencies; C, A dense record in which at least three distinct units may be found; and D, An interference pattern.

spike interval sample size needed to make meaningful determinations of parameters such as mean firing rate. The sample sizes cited in the literature vary widely (2, 3) and evidence will be presented to show that the smaller sample sizes (e.g., Das Gupta and Simpson (2) use four interspike intervals) are not large enough to be meaningful.

There are several reasons why a statistical study of the motor unit spike train is needed. Most important, a stochastic specification will provide meaningful criteria around which to design physiological studies. The need for sample size estimates has been mentioned. In addition, a stochastic description may give an insight into a rather simple nerve-muscle preparation and so aid in model making. Finally, a population of such spike trains constitutes the interference pattern electromyogram, which has been much studied. A detailed knowledge of the statistical makeup of this signal is a necessary guide to its interpretation. For example, a knowledge of the firing frequency range of a single motor unit allows one to estimate how many units are contributing to a given interference pattern.

Before proceeding it is necessary to sketch the statistical framework and present a few definitions. The raw material for a statistical analysis is a sequence of motor unit spikes or action potentials (see Fig. 1). A spike is a complex waveform produced by a number of muscle fibers firing in approximate synchrony; its form depends on the type of electrode used and its position relative to the muscle fibers producing it. The spike is about 8 msec in duration (4, 5). Recorded on film moving at 50 cm per second, this represents a width of 4 mm for the over-all waveform; a single phase (pulse) of this waveform is much narrower. Since all the spikes of a train are very much alike (6), it is easy to pick a point on the waveform as a reference and to use it to locate the waveform. The interval between two spikes (interspike interval, abbreviated ISI) is then the distance between the same reference point on the two spikes. Since the ISI is the statistic of concern, this method of locating a spike relative to another, with its inherent accuracy well in excess of the accuracy of the measuring instrument, is employed. If the shape of the spike itself or its location according to an external frame is of interest, more sophisticated methods of epoch detection are called for (7).

There exists a general class of stochastic processes called the stochastic point process. A paper by Perkel et al. (8) gives a rigorous, lucid, and very thorough treatment of such processes; a brief sketch will be adequate here.

If we start a clock at some arbitrary time and apply the time measure to a spike train, then a unique instant of time may be associated with each spike. The spikes are considered events and each is considered indistinguishable from the others except by its coordinate (point) on the time axis. We have now converted the spike train into a stochastic point process.

"In any point process, in which all "events" (spikes, for example) are indistinguishable except for their times of occurrence, it is the elapsed time between events, e.g. the interspike intervals, that exhibit the properties of random variables. These intervals are regarded as being drawn (not necessarily independently) from an underlying probability distribution (8)."

A renewal process is a stochastic process that "has the property that the lengths of intervals between events are statistically independent (8)."

The tables of ISI's obtained are subjected to a variety of statistical manipulations.

METHODS

Electrical recordings were taken from the brachial biceps of four volunteer subjects. Human subjects were used so that the results could be directly related to studies in clinical and experimental electromyography. The subject was supine on a lightly padded board, the upper arm at his side, and the lower arm flexed so that it was perpendicular to the board. The upper arm was held in a fixed position by a trough-shaped aluminum guide. A cuff about the wrist was connected by a flexible wire to a hardened steel bar on which a strain gage bridge (SR4 Resistance Wire Strain Gage, Baldwin-Lima-Hamilton, Waltham, Mass.) was mounted. Thus, tension could be monitored to assure a stationary output.

Single motor unit activity was recorded using fine wire electrodes modified from a technique of Basmajian (9). Action potentials were amplified and recorded on magnetic tape. The raw data for the determination of the statistical behavior of the single motor unit were in the form of film strip photographs taken when the data on tape were displayed on an oscilloscope. The photographs were made with a Grass Kymograph Camera (Grass Instruments, Quincy, Mass.) at a recording speed of 50 cm per second. At this speed the film came up to speed in 20 msec and stopped in 60 msec. Time marks were generated (Tektronix Crystal-controlled Time Mark Generator, Type 180 A), Roslyn Heights, N.Y. and recorded during each intramuscular recording experiment. These time marks, reproduced on the film strips, were used to calibrate the calipers used to measure interspike intervals. Any constant error in tape or film speed would affect time mark and ISI equally and produce no measured error. The only possible source of error, variations in film speed, proved too small to measure.

A pair of calipers driving a potentiometer was connected to a digital voltmeter and used to measure interspike intervals. The zero setting and a linear scaling factor were adjustable; this permitted aligning the calipers with two time mark pulses and adjusting the scale factor to read the distance between them directly in milliseconds. In this manner all lengths on the film strip were read directly in time units.

To test the accuracy of this device, a synthetic spike train with known ISI's was produced and measured. This allowed determination of the error in measuring each ISI and the cumulative total error. The synthetic spike train was generated as follows. Ten distinct ISI's were used, of length 20 msec, 40 msec, 60 msec, . . . 200 msec respectively. Each appeared in the record 10 times, to produce a total record of 100 ISI's whose order was determined by a random numbers table. The voltmeter as used here could be read to an accuracy of 0.5 msec, and generated an error of $\pm$ 1 msec which occurred at random in about 50 per cent of the readings and was absolute (as opposed to a per cent error). The cumulative error in measuring the test strip was -18 msec; for a test strip of 1100 msec duration, this meant a 0.165% error.

Because of the way instrument error was produced, all ISI's were read to 1 msec accuracy. Measurement produced a table of numbers representing the ISI size of consecutive intervals and these were subjected to statistical scrutiny.

RESULTS

150 such tables were produced. 20 of these had between 30 and 50 ISI's and 35 had over 100. Since the variances of the distributions were not known initially, the largest sample

TABLE I

STATISTICS OF EIGHT TYPICAL MOTOR UNITS

Unit	N	Mean ISI	SD	Mean firing rate
	ISI	*msec*	*msec*	
FM 1-3	50	55.02	8.67	18.18
FM 1-4	73	60.16	7.80	16.62
AF 3-4	93	73.33	9.38	13.65
AF 5-23	98	79.31	7.50	12.61
SM 4-9	94	83.05	15.22	12.05
WL 2-15	114	91.85	12.44	10.89
WL 2-17	104	94.02	8.84	10.64
WL 1-13	79	129.22	21.83	7.74

TABLE II

STATISTICAL PROPERTIES OF THE "LARGE SAMPLE" UNITS

Unit	N	Mean ISI	SD	Mean firing rate
	ISI	*msec*	*msec*	
UR 1-3	567	54.04	5.81	18.52
WL 4-25	379	85.23	13.18	11.73
WL 3-13	232	140.65	29.33	7.12

consistent with experimental accuracy was obtained. The sample had to be of consecutive spikes. Since fatigue has been shown to change motor unit behavior (10), a time limit of ½ min was adopted for record length. These two factors limited sample size.

The firing rates varied between about seven spikes per second and 25 spikes per second. The latter was recorded at maximum voluntary contraction. Some parameters for eight of these units are given in Table I.

Higher order statistical manipulations are particularly susceptible to artifacts due to sample size. In an attempt to avoid this, three spike trains were selected for intensive study (see below, Higher Order Statistics). They were chosen because records were clear for the full 30-sec recording time and showed no evidence of experimental nonstationarity, (e.g. more units "coming in", inability of the subject to maintain constant tension) and because they represented typical units at the extrema and center of the firing range. Some parameters of these three units are given in Table II.

FIRST ORDER STATISTICS

To be considered for meaningful statistical analysis, a motor unit spike train must meet several criteria:

1. Stationarity: There must be no evidence (in graphical display and scatter diagrams) of firing frequency drift. The absence of drift makes of the data a time-invariant process and allows the use of the powerful analytical tools that apply to stationary point processes. Analysis of time variant processes, conversely, is difficult and chiefly designed to determine the nature of that variation, which is not of interest here.

2. Sample size: This criterion is discussed in Results. For statistical evaluation, it is always desirable to collect the most data consistent with experimental design, and the use of fewer than 30 data points makes small-number approximations mandatory in most cases. These in turn are predicated on assumptions regarding the nature of the sampled population and do not aid in determining it.

3. Uniformity of the record: The spike train must be continuously visible. Thus, there are no gaps in the record. This avoids three pitfalls. First, is a gap to be counted as an ISI? Second, may the ISI's on either side of the gap be considered as samples from one population with any confidence? Third, what assurance do we have that the spike trains on either side of a long gap are from the same unit?

136 spike trains meeting these criteria are analyzed.

As may be seen from the histograms of Fig. 2, the ISI distribution takes roughly Gaussian form. One or another histogram may exhibit slight skewness, but a positive or negative skewness is not characteristic of the histograms. They represent, on the whole, a symmetric unimodal distribution about the mean ISI. In addition, the records show an absence of the very long or very short intervals, in agreement with the properties of the Gaussian process (11).

To test the Gaussian hypothesis more rigorously, the cumulative distribution function for each of the eleven units of Tables I and II may be approximated from

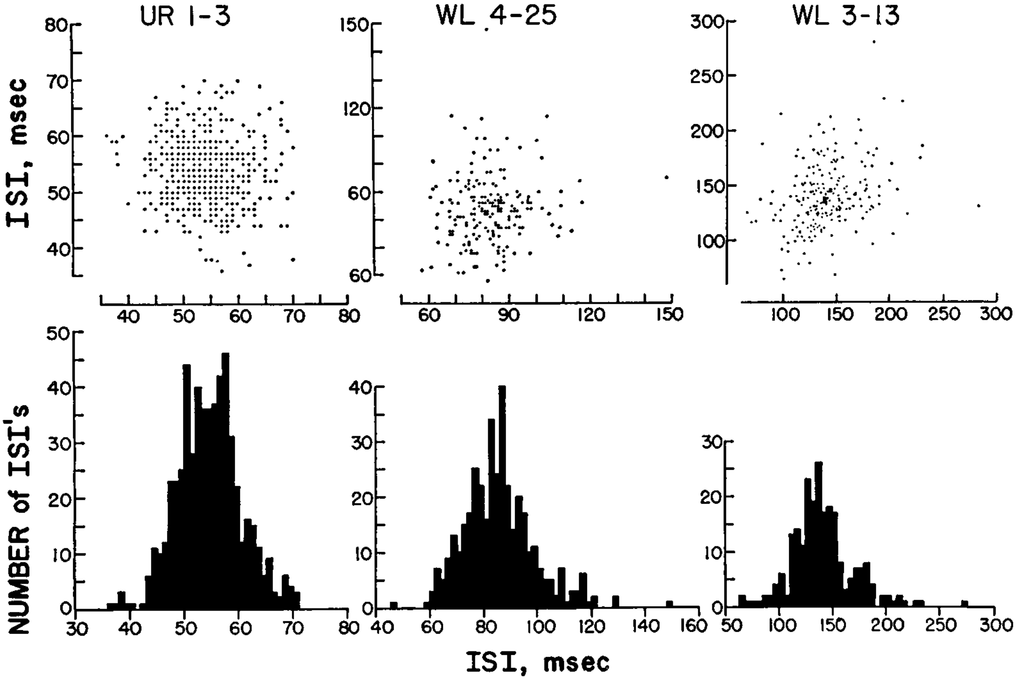

FIGURE 2 Histograms of successive interspike intervals, and corresponding scatter diagrams, for three "large sample" units. Bin width is in milliseconds for UR 1-3 and WL 4-25 and 5 msec for WL 3-13.

the respective histograms. The cumulative distribution function is

$$F(\tau) = \int_0^\tau f(t)\,dt = p(T \leq \tau)$$

and may be approximated by reference 8

$$F_j = \sum_{k=1}^{j} f_k .$$

This is accomplished in practice by summing the contents of j bins in the histogram. The result is a monotone increasing sequence of integers, the number of ISI's of value $\leqq j$. The sequence is normalized, so that $0 \leqq F_j \leqq 1$ as required, and plotted against j on probability scale graph paper. If the ISI's are normally distributed, the points will lie in a straight line. Some plots and straight line fits are shown in Fig. 3. The goodness of fit to these "straight lines" (which represent normal distributions) may be determined by the chi-square test. For about 90% of our units, the hypothesis of normality may be accepted at a level exceeding 99.5%. Thus, for nearly all units, the hypothesis that the underlying distributions are gaussian may be accepted with considerable confidence on the basis of first-order tests.

To determine if successive interspike intervals are statistically independent (for the case of a gaussian distribution, this is equivalent to uncorrelated), we employ the joint interval density plot. This is a graph of points generated by considering an interspike interval as the abcissa of the point and the next interval as its ordinate. An inspection of these plots allows us to distinguish two possible models for the motor unit. First, an "absolute clock" could determine the unit's firing rate with the unit responding to each clock command after a delay governed by a gaussian distribution. This implies a relation between successive interspike intervals. Clearly, long intervals must be followed by short ones to keep the unit in synchrony with the clock. The second possibility is that the probability of firing is gaussian distributed with a mean $\bar{x}$ in time after the last firing. Such a model is represented by a renewal process. If an "absolute clock" governs a motor unit and long intervals tend to be followed by short ones, the points will cluster about a straight line with negative slope (negative correlation). If the unit's firing rate shifts toward a different frequency, or even oscillates, this will be reflected in the joint interval density by a tendency for the points to cluster about a line with positive slope (long intervals followed by longer ones or conversely, which is positive correlation). Joint interval densities of three units are shown in Fig. 2. The points lie in a nearly circular region in all cases, suggesting no correlation between successive ISI's and hence, under the gaussian assumption, statistical independence of ISI's.

To further specify the behavior of the spike trains, a plot of the mean vs. standard deviation of the ISI's for each unit is prepared (Fig. 4) along with the best linear fit (linear regression line) to the data.

$$y = 0.175x - 3.54$$

The points show a clear-cut positive correlation, but the exact nature of the relationship is far from clear. The standard deviation increases monotonically, as does the scatter in the data. Both linear (14, 15) and nonlinear (16) curves have been fitted to data of this type. The linear regression line gives the best fit over all; the error in the fit is greatest at the extrema (40 to 60 msec and 120 to 140 msec) and is of like sign in both cases. This suggests that a nonlinear function with monotone increasing slope might provide a better fit. Such a function, relating the mean and standard deviation in the firing pattern of motor units, reduces the description of the motor unit to a one-parameter system. It can also be used to determine the sample size of ISI's required for meaningful analysis, as has been done in the Appendix. To this end, it is most important that the function provide the best fit at the extrema (40 to 60 msec and 120 to 140 msec) since accurate data are abundantly available for the mid-range, and extrapolation of the curve is most meaningful at the high frequency end if the fit is best in that region. To this end, a simple parabolic fit is

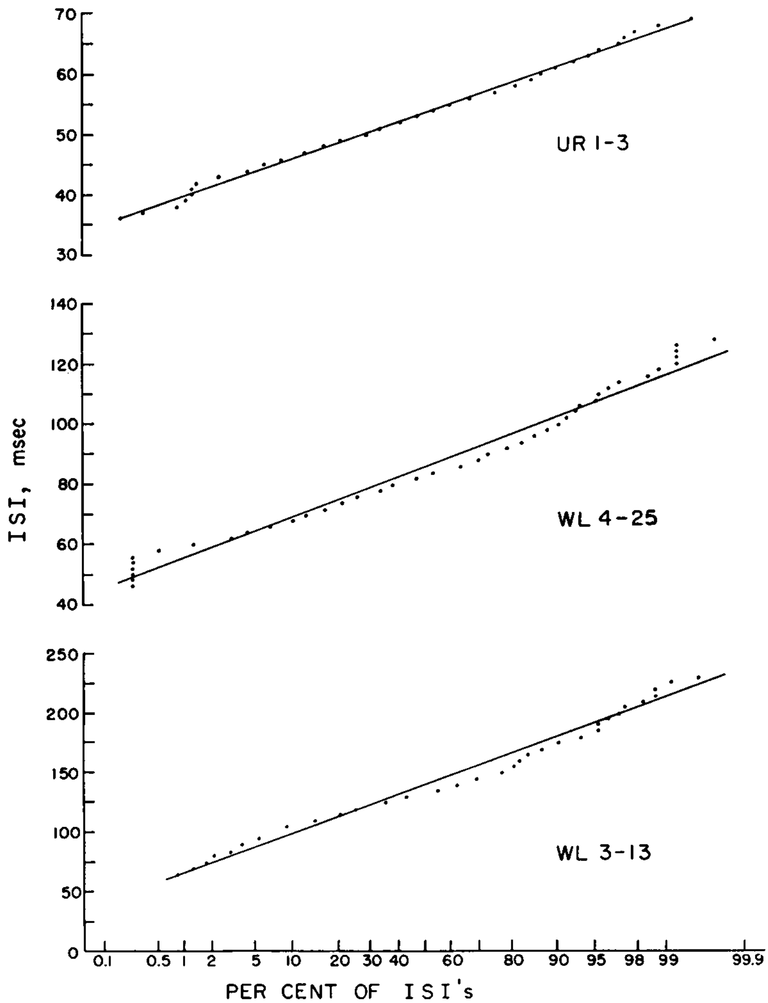

FIGURE 3 Cumulative distribution function and gaussian fit for the "large sample" units displayed on a probability scale (see text).

made. The arithmetic mean (abscissa and ordinate) coordinate (center of gravity) is determined for the cluster of points lying on the intervals

$$x \leqq I \leqq x + 10 \text{ msec}, x = 50, 100$$

and a curve of the form

$$y = ax^2 + b$$

adjusted to fit these points. This curve, shown in the Appendix, is used there to

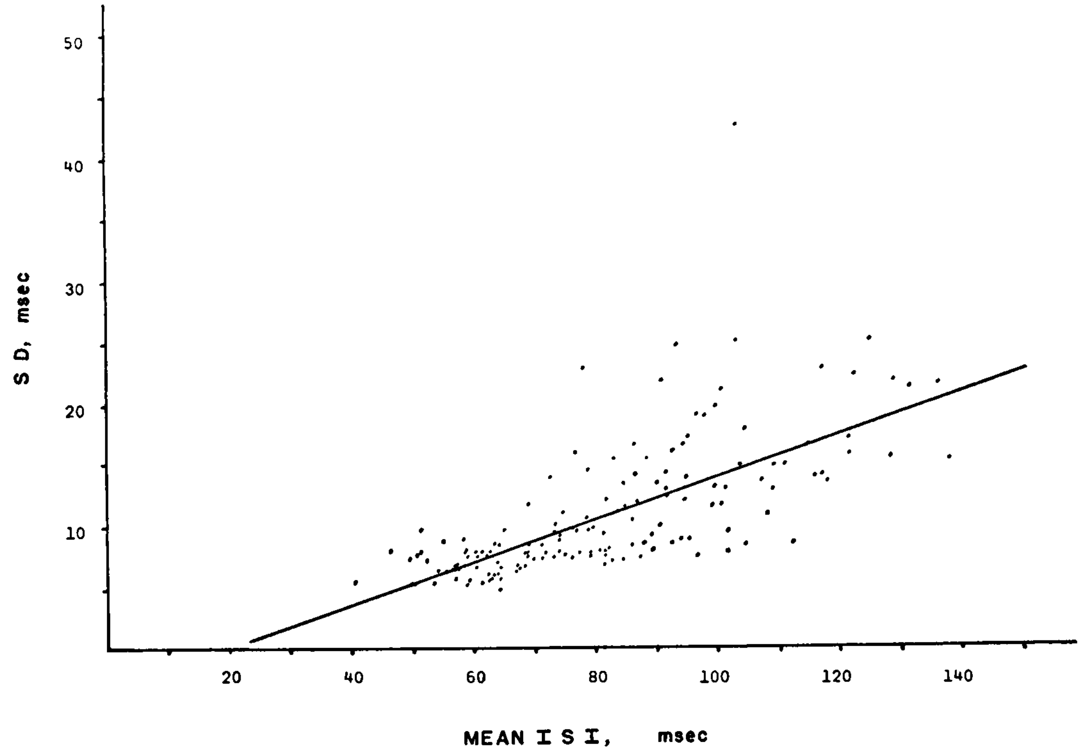

FIGURE 4 Plot of mean ISI against standard deviation with linear regression line for 150 units.

determine sample size requirements. These requirements, extrapolated to the region 40 to 60 msec, show that a sample size of 10 is entirely adequate for accurate estimation of the mean. This interval corresponds to very high muscle effort which makes recording single units and the maintenance of stationarity most difficult. Fourteen spike train records having a time duration of between 10 and 15 sec and in which movement artifacts produced momentary breaks have been analyzed. The number of interspike intervals in these records is too low to satisfy condition 1 above for acceptable spike trains, but it is shown in the Appendix that their sample size is entirely adequate for a rapidly firing unit. Analysis of these short records produced data in perfect agreement with prior results on longer (30-sec) records.

The firing pattern of a neuron may be represented mathematically as a random walk with drift (11). In this view, the membrane potential drifts from a starting point and "wanders", as a random walk process biased in favor of one direction, until it reaches the threshold-of-firing level. At this point, a spike is produced and the membrane potential is returned to its starting point. In this model, the firing rate is increased by increasing the drift force. But this reduces the length of time during which the random process is effective, i.e., reduces the weight of the effect.

Thus one would expect greater scatter in data collected from a slowly firing neuron than in a rapidly firing one, if both are governed by this model. At the extrema, the infinitely strong drift would produce zero scatter and it is well known that the case of zero drift produces a random process with scatter so large that it has no moments of any order. The distribution of Fig. 4 suggests that a random walk model is certainly intuitively appealing.

We may summarize the preceding treatment as follows. The motor unit firing pattern may be modelled by a stochastic renewal point process, the underlying probability density of the jitter being gaussian. The mean and standard deviation of the probability density are functionally related, making it possible to completely specify the process by determining its mean. These results may be tested and expanded by an examination of some higher order statistics.

SOME HIGHER ORDER STATISTICS

The results section mentions the selection of three representative units for further analysis; these units, because of the large sample populations, are discussed here. The operations performed on them are the obtaining of autocorrelograms and some manipulations with grouped interval histograms. All these manipulations refer to trains of identical events treated as impulse function trains; i.e., to manipulations on stochastic point processes. The analog data recorded on tape were not so treated as the influence of spike shape may be regarded as an artifact due to recording needle configuration and position and is irrelevant to this treatment.

We may view the autocorrelogram of a train of events as the process of generating a duplicate record and "sliding one record over the other" (14). The autocorrelogram is nonzero only at those points in time when two events overlap during this sliding process. But this overlap is just the time distance (interspike interval) between those two events. The autocorrelogram may thus be generated by noting first all adjacent interspike intervals; second, the sums of all possible pairs of adjacent interspike intervals; and third, the sums of all possible triples, and so on (17). In a record of finite length, the generation of an autocorrelogram involves manipulation with two identical trains of spikes, and hence spike trains of equal length. As one is slid over the other, its end extends into a region of no record, i.e., of all zeroes. Spikes in the "moving" record cannot come into coincidence with spikes on the fixed record because there are none. This produces an artifact: the decline in the maximum possible value in the autocorrelogram with increasing time. An example may make this clearer. Suppose that the autocorrelogram of a train of equally-spaced impulse functions is sought. In theory, this is a train of equally spaced pulses of unit weight. In practice, suppose we have a record of 100 of these spikes. The "sliding" algorithm produces a value of 1 for the autocorrelogram at $t = 0$; the next nonzero value will be 0.98, and so on. To minimize this effect, the record should be long in comparison to the individual ISI's, (3×10^4 to 141 at worst here) and the length of the autocorrelogram should be short compared to the length of the record.

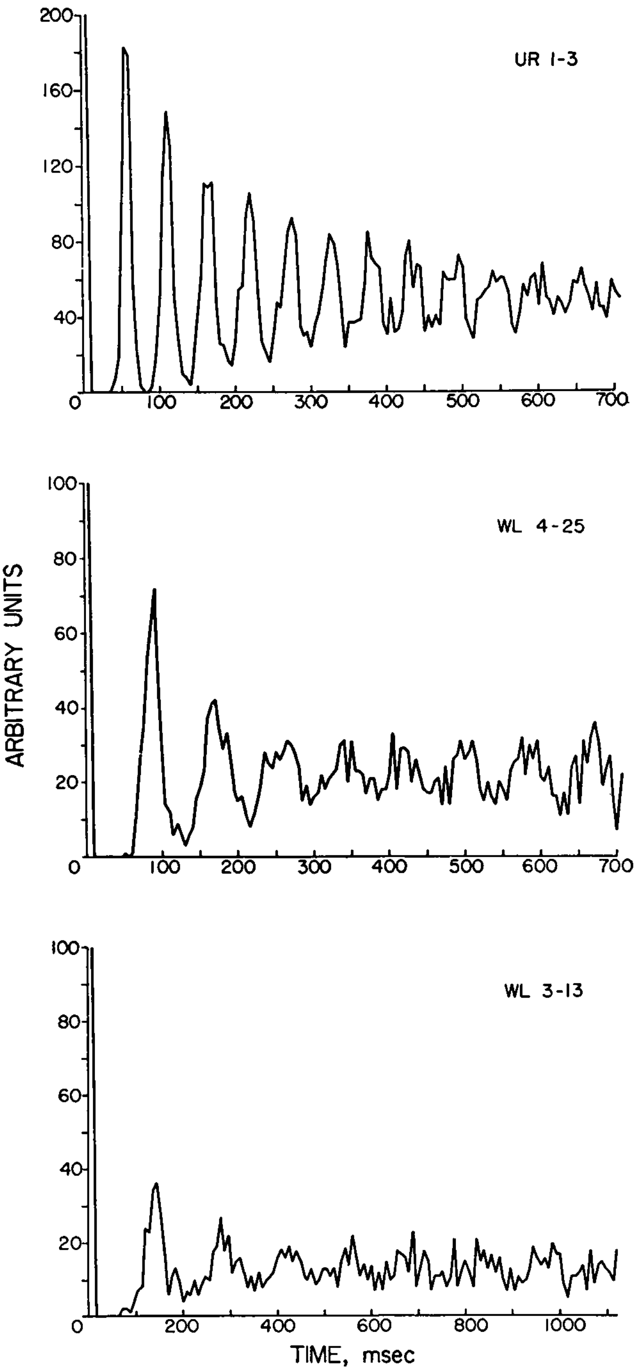

FIGURE 5 Autocorrelograms for the "large sample" units.

The numbers generated by the above process are assigned to bins of an autocorrelation histogram. Autocorrelograms of three units are shown in Fig. 5. An absolute clock model yields a periodic correlogram with peaks of constant amplitude, as in the example just given, while a renewal process generates an autocorrelogram that resembles a damped oscillator. The latter is a far better fit to the results, reinforcing the hypothesis of statistical independence. The greater the standard deviation in the underlying probability density, the greater is the rate of damping. The autocorrelogram should finally "level off" at the mean ISI (14).

An interesting test can be applied to the interspike intervals to determine how closely they approach the gaussian ideal. There is a limited class of functions, called stable by Gerstein and Mandelbrot (11) which have the property that convolution of the probability density function with itself n times gives back the same function, with only a change of scale. The gaussian distribution is one of these; n convolutions of the gaussian distribution (m, σ) with itself yields a gaussian function $(nm, \sqrt{n}\sigma)$.

A digital computer program has been written to produce an algorithm for this process, by generating grouped interval histograms. These are histograms of successive sums of n numbers of the original data and are called scaled interval histograms in reference 11. Inspection of the grouped interval histograms so generated shows that there is a qualitative change of shape. Successive histograms become more peaked, as if the gaussian distribution were giving way to an exponential distribution of the form exp $a \mid t - b \mid$. Histograms resulting from more than 15 convolutions begin to show slight skewness, with large numbers being more likely than small ones. The histograms are approximately scaled, since the exact scaling factor is meaningful only for a stable function. More conclusive evidence are graphs of the mean and standard deviation of the results of the n'th convolution vs. n. The scaling factor for m agrees remarkably with the prediction; the scaling factor for σ however, predicted by theory to be the $\sqrt{n}$, is more nearly n^2.

The close agreement of the location of the means with prediction is simply a result of the near-perfect symmetry of the data. The plot of the actual data lies slightly below the theoretical curve. This suggests a slight skewness, with very long intervals being slightly more likely than very short ones. The phenomenon is most marked in the unit of slowest firing frequency; this is intuitively appealing since a unit firing at its threshold of recruitment could be expected to "drop out" and "come in", generating very long interspike intervals. Statistical methods cannot distinguish temporary cessation of firing from long interspike intervals; the observer can only discriminate between the two by definition. It should be noted that the skewness in all cases is very slight; in the worst case, five successive convolutions produce a deviation from the result predicted by symmetry of less than 2%.

According to this extremely strict test, ISI's are not gaussian distributed, but even here a high degree of symmetry for the underlying probability density is shown. The gaussian description, though not perfect, may be retained with considerable con-

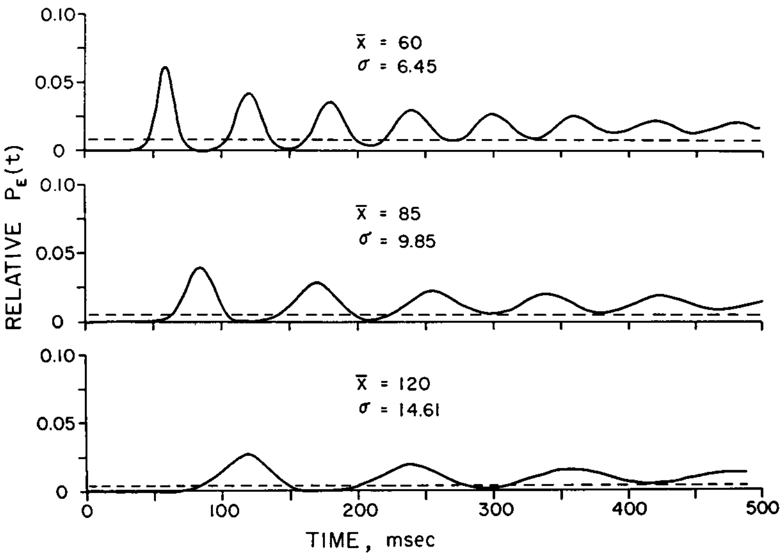

FIGURE 6 Probability of firing after a spike at $t = 0$ for three hypothetical firing frequencies.

fidence on the strength of the first-order tests. The small inaccuracy that may be introduced is a small price to pay for the resulting simplicity of the model.

The stability property of the gaussian process is the basis for the following fact. If a spike in a train is known to occur at $t = 0$, the probability of the occurrence of the next one at any time t is given by a gaussian process with mean m, standard deviation σ. The probability that the next (second) will occur at some time t is given by a gaussian process with mean $2m$, and standard deviation $\sqrt{2}\sigma$, and so forth. Thus, the location of the n'th spike is given by a gaussian function with mean nm, and standard deviation $\sqrt{n}\sigma$. The probability of occurrence of a spike at any time after a known event has occurred is given for three typical firing rates in Fig. 6.

The maximum probability of occurrence of the n'th spike is at time nm and has the value

$$p_{\max}(t) = \frac{0.3989}{n^{1/2}}$$

Thus the maximum probability of occurrence of a given spike varies inversely as $\sqrt{n}$, assuming σ constant. Let a train of such impulses be divided into intervals of length m and added. The i'th such interval contains the point of maximum probability of occurrence of the i'th spike, a fixed distance from one end. Likewise, the j'th such interval contains the point of maximum probability of occurrence of the j'th spike, in the same place relative to one end. Superposition of these intervals will cause a concomitant superposition of the probabilities; the probability of a spike occurring in a record composed of n such segments is the sum of the individual probabilities

$$p_{\text{total}}(t) = \sum_{j=1}^{n} p_{\max}(t) = \sum_{j=1}^{n} \frac{0.3989}{\sigma j^{1/2}} \frac{0.3989\sqrt{n}}{\sigma}$$

So the probability of occurrence of a spike in a given place on the summed record increases as the square root of the number of segments added into it. For the case where a large number of segments are added to form the summed record, this is equivalent to saying that the maximum amplitude of the result increases as $k\sqrt{n}$, where k is a constant and n the number of intervals summed. If the unit is in an interference pattern, all other spikes occur at random with respect to the one under study. Superposition of such spikes (regarded as noise) also produces an amplitude increase of $\sqrt{n}$, for a signal to noise ratio of unity. In other words, an attempt to bring a motor unit out of an interference pattern by averaging is doomed to failure even if its mean firing rate is known a priori, which it cannot be.

A graphical result is the following: define a "coefficient of variation" as $p_f(t_1)$ where $t_1 = m + 2\sigma$. Since $\sigma = \sigma(m)$, it may be seen that an event cannot be located 19 times out of 20 (probability of error 5%) after the fourth firing for $m = 60$, and after that, the pulse can occur at any time with a 95% or higher probability (See Fig. 6). And this is the gaussian assumption. The actual result as discussed in the section on grouped interval histograms, is far more dismal since σ increases far more rapidly under convolution than by $\sqrt{n}\sigma$. In short, once a spike is located, no predictor can be expected to give a reliable result for more than the next three spikes.

It is tempting to think that a detailed knowledge of the statistics of motor unit firing patterns will allow the design of a predictor that can find the spikes belonging to one unit in the "noise" of an interference pattern. We can now see that this is not so. The calculations produce the lesser result that such a predictor is no better than a random guess after three spikes. The definition of the interference pattern precludes a general method of finding spike trains by a method of shape recognition. However, a combination would use the predictor to locate "the next spike", with confirmation by some shape criterion, and use the newly located spike to find the next, and so forth in an iterative procedure.

We may summarize the preceding treatment as follows. A motor unit spike train readily lends itself to a variety of statistical manipulations. A first-order model of the motor unit as a stochastic renewal point process with gaussian jitter is derived from this analysis. A relationship between σ and μ in this model allows specifying the model by one parameter. It follows from the model that for the muscle under study, a sample size of 50 interspike intervals is needed in most cases to derive meaningful ISI statistics. Such a criterion is needed to assure that studies of motor unit parameters do not suffer from small sample errors. Higher order tests specify the spike train more precisely and show that a stable probability density function does not represent a good model of the data at this level of analysis.

APPENDIX

Introduction

The most obvious statistics to be obtained from a train of motor unit spikes are the mean interspike interval, its reciprocal, the mean firing frequency, and the variance (or standard deviation) of the interspike interval size about the mean. Any statement about these statistics must be tempered by the fact that they are sample statistics and not necessarily the true properties of the population under study. Here we wish to answer the question: How large must the sample size N of a motor unit spike train be to estimate the population mean with sufficient accuracy? The criteria found will determine what we mean by the term "sufficient".

Assumptions and Notation

The firing pattern of a single unit is assumed to be a gaussian process with mean μ and variance σ^2; these are functionally related by

$$\sigma = 9.1 \times 10^{-4}\mu^2 + 4.0 \tag{1}$$

This relation and the coefficients were empirically determined from observed data (Fig. 7). The process is further assumed to be stationary, and successive interspike intervals are statistically independent.

The notation is relatively standard. The observed event X is a single interspike interval. The sample mean and variance are respectively denoted by μ and σ^2 and the variance of the sample mean is called s^2.

Statistical Considerations

Our sample consists of a time series of interspike intervals: $X_1, X_2, \cdots X_N$. This has a

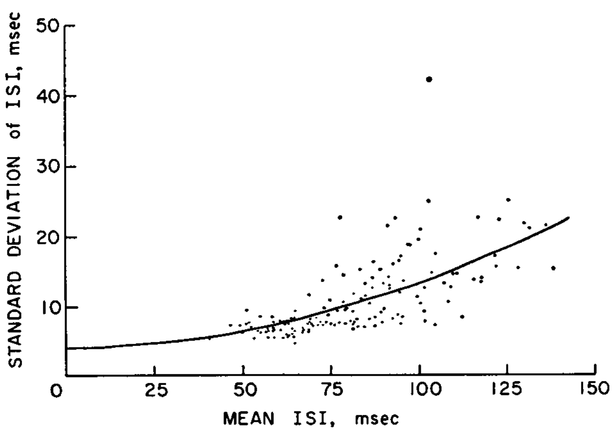

FIGURE 7 Mean vs. standard deviation for 150 units, with quadratic fit to the data.

mean value (sample mean)

$$\mu = \frac{1}{N}\sum_{i=1}^{N} X_i \tag{2}$$

Now, in general, the second moment of a random variable is

$$E[X^2] = \int_{-\infty}^{\infty} x^2 p(x)\, dx \tag{3}$$

Hence

$$E[kx^2] = \int_{-\infty}^{\infty} (kx)^2 p(x)\, dx = k^2 E[X^2]. \tag{4}$$

The variance of the sample mean μ is

$$\text{var}\,[\mu] = \text{var}\left[\frac{1}{N}\sum_{i=1}^{N} X_i\right]. \tag{5}$$

Using equation 4

$$\text{var}\,[\mu] = \frac{1}{N^2}\,\text{var}\sum_{i=1}^{N} X_1.$$

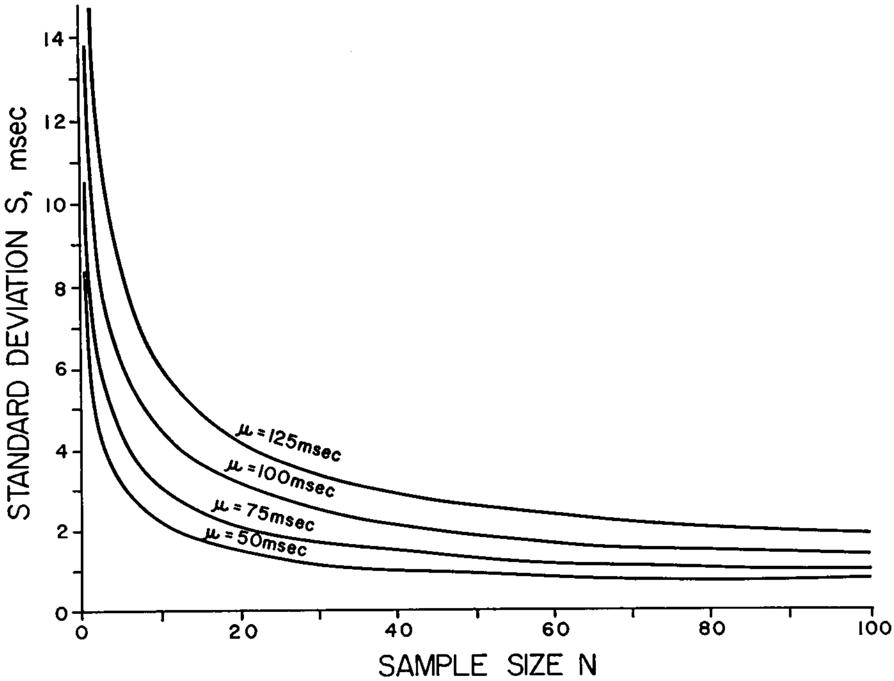

FIGURE 8 A plot of s vs. N for four constant values of μ.

Since the X_i are statistically independent,

$$\text{var}\left[\sum_{i=1}^{N} X_i\right] = \sum_{i=1}^{N} \text{var } X_i = N^2. \tag{6}$$

Hence

$$\text{var } \mu = s^2 = \frac{\sigma^2}{N}. \tag{7}$$

This result, which is quite straightforward, will now be used to determine the sample size needed to attain a given accuracy for the mean interspike interval. For the case at hand,

$$s = \frac{\sigma}{N^{1/2}} \frac{a\mu^2 + b}{N^{1/2}} \tag{8}$$

where

$$a = 9.1 \times 10^{-4}$$
$$b = 4.0$$

are empirically determined constants.

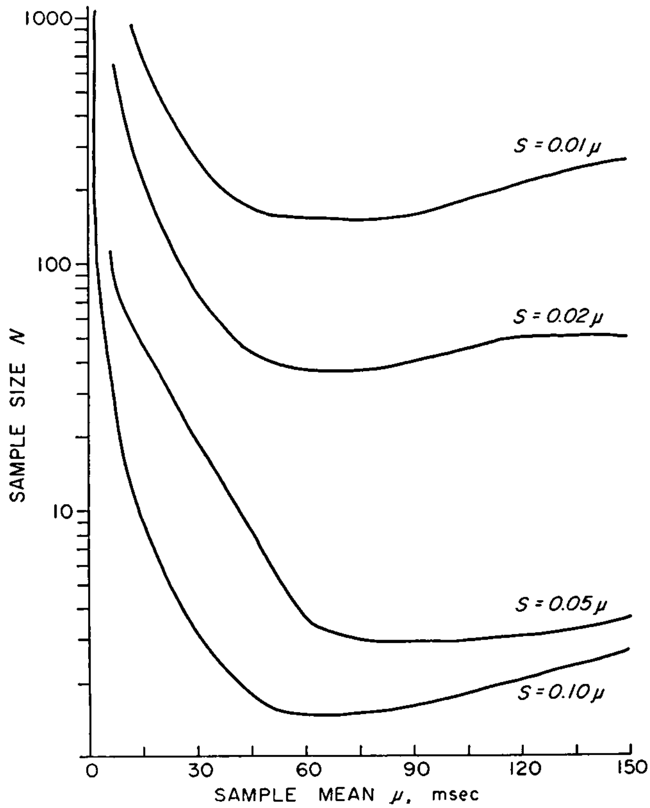

FIGURE 9 N as a function of the sample mean μ, when the standard deviation of the mean is chosen as a fixed fraction of μ.

Thus, for a fixed sample size N, we have a quadratic relation between the standard deviation of the sample mean and the sample mean. To maintain the standard deviation of the mean constant, the sample size must increase as the fourth root of the mean (population) interspike interval.

The three figures display this relation in different ways. Fig. 8 is a plot of s vs. N for four constant values of μ. Fig. 9 shows N as a function of the sample mean μ, when the standard deviation of the mean is chosen as a fixed fraction of μ. The last figure (Fig. 10) shows N as a function of μ for several fixed values of s in milliseconds.

For 95% of the sample means to be within $\pm$ 4% of the true mean, a sample size of about 50 is required over most of the physiological range (μ = 46 msec to 140 msec). If we are satisfied with 95% of the sample means lying within $\pm$ 10% of the population mean, then sample sizes of 20 (μ = 30) to 3 ($75 \leq \mu \leq 120$) are adequate. These considerations affected the design of several experiments intended to determine the maximum frequency of firing of a single motor unit. Records were taken near maximum voluntary contraction, and searched for rapidly firing (μ = 40 to 60) motor units. Although such a search is difficult in such

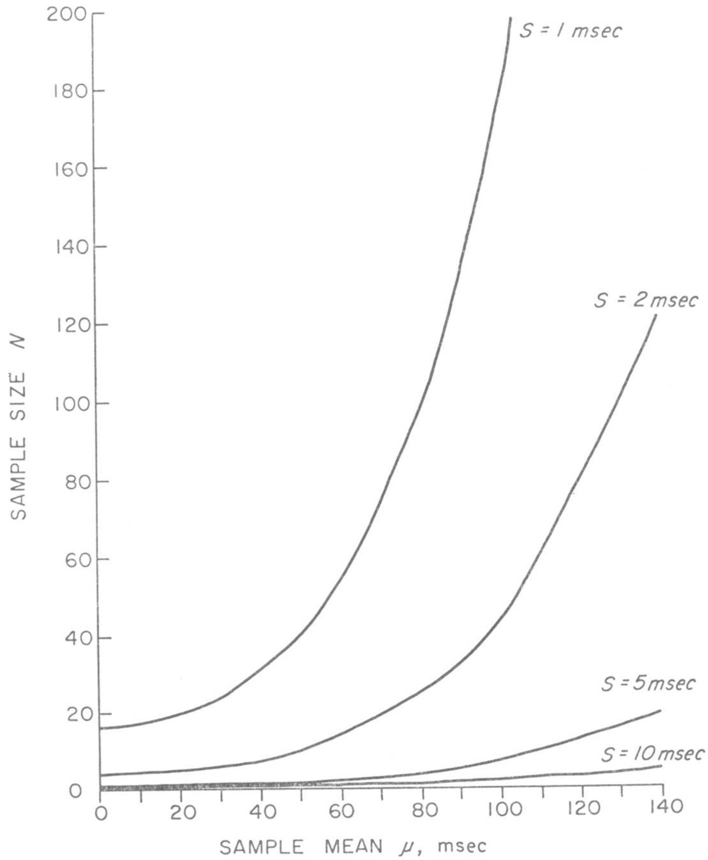

FIGURE 10 N as a function of μ for several fixed values of s in milliseconds.

crowded records, 14 single unit traces could be located; as expected, however, there were large sections of the record where single units could not be identified. Hence, the records read were of necessity interrupted and the sample size small. But the units we hoped to find had $\mu = 40$ to 60 so that a sample size of at least 10 would put μ within $\pm$ 4 msec of the population mean 95% of the time. This was considered satisfactory. In practice, only one unit ($\mu = 51.50$) had $N = 10$; the remaining sample sizes ranged from 22 to 99, well within the limits of twice that accuracy.

Finally, a word should be said about the relationship

$$\sigma = 9.1 \times 10^{-4}\mu^2 + 4.0$$

Data from 136 motor units were averaged over blocks in mean ISI of 10. Thus e.g., the μ and σ values of all units such that $60 \leq \mu \leq 70$ were averaged. This resulted in nine points, each representing the average of between 3 and 24 individual spike trains, or between 110 and 2200 interspike intervals per point. This sufficiently exceeds the accuracy criteria determined to be adequate to answer charges of "bootstrapping", i.e., using the data to determine the accuracy of the same data. The only assumption required is stationarity of the data.

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