INFLUENCE OF THE LATENCY FLUCTUATIONS AND THE QUANTAL PROCESS OF TRANSMITTER RELEASE ON THE END-PLATE POTENTIALS' AMPLITUDE DISTRIBUTION

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ABSTRACT Spontaneous synaptic potentials and their relation to the end-plate potential (e.p.p.) are studied. It has been suggested earlier that the e.p.p. at a single nerve-muscle junction is built up statistically of small all-or-none units which are identical in size with the spontaneous miniature end-plate potentials (m.e.p.p.'s). In this paper, a more general theory is developed which takes into account latency fluctuations of the unit components. A general equation for e.p.p. amplitude probability distribution is derived. This probability distribution is a function of the latency distribution, m.e.p.p.'s pulse shape, m.e.p.p.'s amplitude distribution, and the mean quantal content. The time course of transmitter release, or latency distribution, is derived from a histogram of synaptic delays in a frog muscle, but obtained equations can be used for other distribution functions as well.

INTRODUCTION

The elegant experiments of del Castillo and Katz (1954) on the frog neuromuscular junction and of Boyd and Martin (1956) on mammalian muscle, together with work on a number of other preparations, make it abundantly clear that transmitter is released in packets of an approximately constant size, that the packets are released in an all-or-none fashion, and that the total amount of transmitter released must be some integral multiple of the least unit. Furthermore, release of packets is not a deterministic phenomenon, but rather is probabilistic; thus the number of packets released by a nerve impulse varies in a random fashion from impulse to impulse and, in some instances, transmitter release fails to occur.

Suppose that the myoneural junction in each muscle fiber contains a large population of excitable units, each unit capable of responding to a nerve stimulus by producing a unit potential, or quantum, similar to spontaneous potential. Suppose further, that during a magnesium block the probability of such a response from any given unit is small. Then the number of quanta which make up the e.p.p. should fluctuate in a manner described by Poisson's law (for a complete discussion see del

Castillo and Katz, 1954). The mean quantum content m may be obtained by dividing the mean amplitude of a series of e.p.p.'s recorded from the fiber by the mean amplitude of the spontaneous potentials. Once m is known, the theoretical distribution of the e.p.p. amplitudes can be calculated from the mean v and variance σ^2 of the spontaneous amplitude distribution.

Fig. 1 shows the results of the experiment by Boyd and Martin (1956) in mammalian skeletal muscle (cat). 200 e.p.p.'s have been recorded from a single fiber in which neuromuscular transmission was blocked with magnesium. The e.p.p. amplitude ranges from 0.3 to 3.0 mv and peaks occur in the distribution at 0.4, 0.8, 1.2, and 1.6 mv, i.e. at 1, 2, 3, and 4 times the mean amplitude of the spontaneous potentials. To match the experiment with the theory, expected numbers of e.p.p.'s in each quantal group are calculated from the Poisson equation. A gaussian curve is then drawn for the first group about a mean amplitude v and with a variance σ^2. The second group is distributed similarly about a mean of $2v$ and with the variance $2\sigma^2$, and so on for the remaining groups (for complete discussion see Martin, 1966). The individual curves, when added together, give the distribution shown by the continuous curve in Fig. 1. Such curve fitting is based on the assumption that the quantal release is instantaneous and coincident and that the spontaneous potential amplitude distribution is gaussian.

Recent measurement by Katz and Miledi (1965) shows that the quantal release at the neuromuscular junction is not instantaneous, but rather fluctuates in a random way. Also, measurements by Martin and Pilar (1964) show that spontaneous

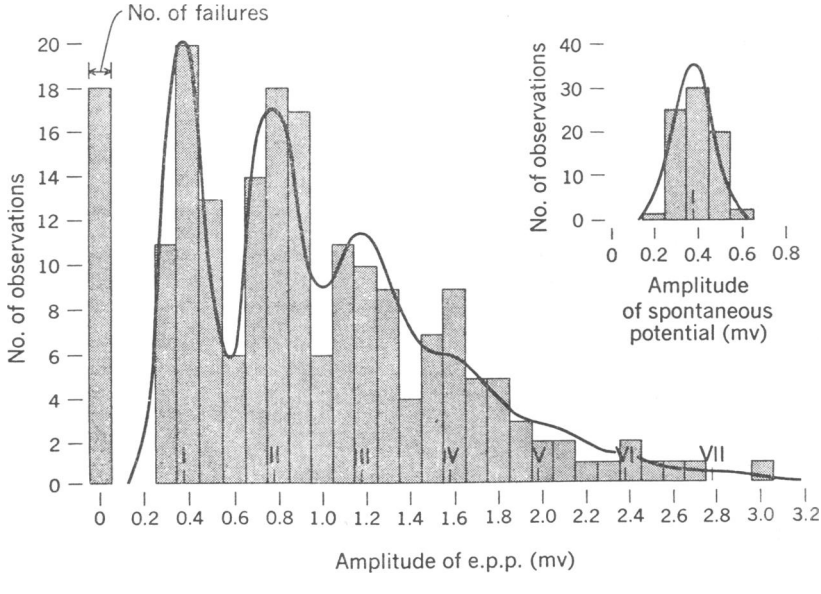

FIGURE 1 Histograms of e.p.p.'s and spontaneous potential amplitudes (inset) from a mammalian end plate (from Boyd and Martin, 1956).

potential amplitude distributions can be skewed rather than gaussian. Here we shall develop a more general theory taking into account random fluctuation of release times, skewed distribution of spontaneous potentials, and influence of pulse shapes of m.e.p.p.'s.

THEORY

In a resting terminal, transmitter packets are released at random intervals with a low probability. It was first shown by del Castillo and Katz (1954) that the number of units released by a nerve impulse arriving at the neuromuscular junction follow a Poisson distribution. This result has since been confirmed by a number of investigators at various synapses. When the terminal is depolarized by an action potential, the release rate $\alpha(t)$ rapidly increases to a high value and then returns to the resting level.

The time course of $\alpha(t)$ at the neuromuscular junction was measured by Katz and Miledi (1965) and results are shown in Fig. 2. The same unit potentials of, say, 0.25 msec rise time and 0.5 mv size can appear as early as 0.5 msec and as late as 2.6 msec after the arrival of the nerve impulse. This indicates that the nerve impulse is followed by a period of a few milliseconds during which the probability of quantal release is increased as shown in Fig. 2. The latency fluctuations can, therefore, be

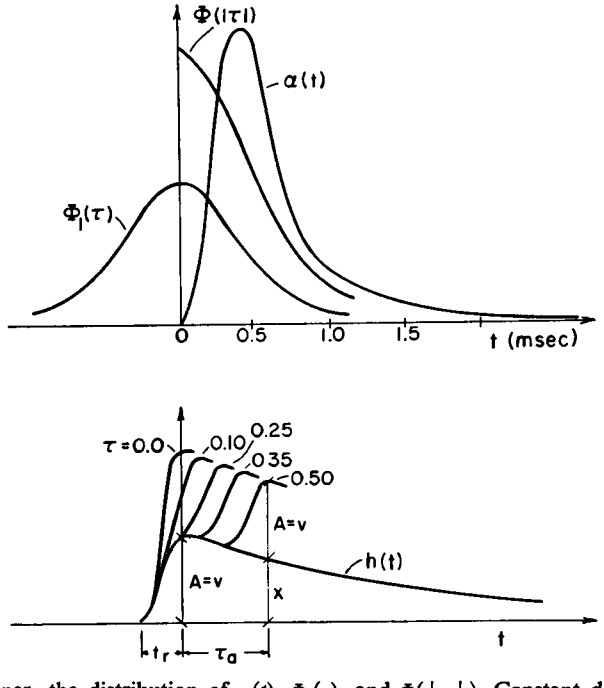

FIGURE 2 Upper: the distribution of $\alpha(t)$, $\Phi_1(\tau)$, and $\Phi(|\tau|)$. Constant delay of ~0.5 msec for release rate $\alpha(t)$ is not shown, because it does not influence the distribution $\Phi(|\tau|)$. Lower: building of e.p.p. pulse from two m.e.p.p.'s with relative delays from 0 to 0.5.

explained as a statistical consequence of the quantal mechanism of transmitter release.

Our goal is to obtain an expression for amplitude distribution function $f(s)$ of e.p.p.'s. The amplitudes s of e.p.p.'s, on the basis of quantal theory, are composed of a sum of m.e.p.p.'s whose amplitudes we shall denote by A. m.e.p.p.'s amplitudes A are randomly distributed with distribution function $P(A)$. It has been shown experimentally that the number of m.e.p.p. units composing an e.p.p. is Poisson distributed. Hence, the probability distribution of the e.p.p.'s amplitude s, which is composed of 1, 2, 3 $\cdots$ k m.e.p.p.'s, will be

$$f(s) = \frac{e^{-m}}{0!}\cdot g_0(s) + \frac{e^{-m}}{1!}\cdot m^1\cdot g_1(s) + \frac{e^{-m}}{2!}\cdot m^2\cdot g_2(s) + \cdots \frac{e^{-m}}{k!}\cdot m^k\cdot g_k(s). \quad (1)$$

We have inserted into the Poisson distribution the functions $g_0(s) \cdots g_k(s)$, which will be used to describe different ways of summation of the m.e.p.p.'s into e.p.p.

The first term of equation 1 describes the number of failures to responses. For such cases $s = 0$, hence

$$g_0(s) = \delta(s). \quad (2)$$

The function $\delta(s)$ is used to denote that the function is different from zero only in one point, in this case for $s = 0$.

The second term describes the cases when e.p.p. response is composed of only one m.e.p.p., hence s and A will have the same probability distribution function

$$g_1(s) = P(A)*\delta(s) = P(s). \quad (3)$$

The third term describes the cases when e.p.p. response is composed of two m.e.p.p.'s added to each other, Fig. 2, lower part. One m.e.p.p. is added with full amplitude A, the other one with fractional amplitude x because of the delay between them. Fraction x has a distribution function $g(x)$ which will be derived later.

As $s = A + x$, its distribution function $g_2(s)$ can be obtained according to the theorem for the sum of two independent stochastic processes, (see e.g. Papoulis, 1965, p. 189).

The sum will have the value s, if one variable has the value A, and the other one the value $x = s - A$. The probability of having the above combination is $P(A)\cdot g(x) = P(A)\cdot g(s - A)$. As A can have any value between $+\infty$ and $-\infty$, there will be a large number of combinations, forming the value s. Probabilities of all those combinations, when integrated, form the probability of s:

$$g_2(s) = \int_{-\infty}^{\infty} P(A)\cdot g(s - A)\, dA. \quad (4)$$

The integral of this kind is known as a convolution (*),

$$g_2(s) = P(A)*g(x). \tag{4a}$$

In a similar way, one can conclude

$$g_3(s) = P(A)*g(x)*g(x), \tag{5}$$

$$g_k(s) = P(A)*g(x) \cdots *g(x) = g_{k-1}(s)*g(x). \tag{6}$$

Using equations 1 and 6 we can describe different models for building e.p.p.'s from m.e.p.p.'s. We shall start with the simplest model and gradually take more and more parameters into account going toward more realistic models.

(*a*) *Constant amplitudes v of m.e.p.p.'s and immediate response.* For this simple case we have

$$P(A) = \delta(A - v), \qquad g_1(s) = P(s - v),$$

$$g(x) = \delta(x - v),$$

$$g_k(s) = \delta(s - kv),$$

$$f(s) = \frac{e^{-m}}{0!}\delta(s) + \cdots \frac{e^{-m}}{k!}m^k \cdot \delta(s - kv). \tag{7}$$

Poisson distribution given by equation 7 is shown in Fig. 3 *a*.

(*b*) *Arbitrary distribution of m.e.p.p.'s and immediate response.* For this case we have $x = A$ and

$$g_1(s) = P(s),$$

$$g(x) = P(x), \tag{8}$$

because each m.e.p.p. is added with full amplitude. Hence,

$$g_k(s) = P(A)*P(x) \cdots P(x). \tag{9}$$

This expression is valid regardless of the form of $P(A)$ which can be normal or skewed. For normal distribution we have

$$g_1(s) = \text{normal } (v, \sigma),$$
$$g(s) = \text{normal } (v, \sigma).$$

The distribution $g_k(s)$ will now be a multiple convolution of normal distributions.

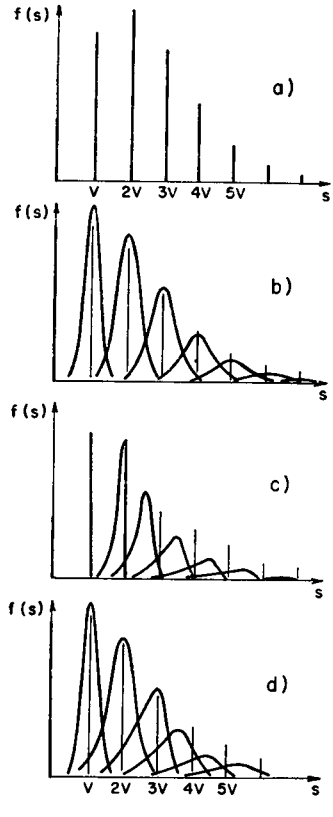

FIGURE 3 The distributions of e.p.p.'s amplitudes for four different models.

The convolution of the normal distribution (v, σ) by itself produces again normal distribution $(2v, \sqrt{2}\,\sigma)$. (See e.g. Papoulis, 1965, p. 266). After k convolutions, we have

$$g_k(s) = \text{normal } (kv, \sqrt{k}\,\sigma), \tag{10}$$

according to the law for addition of normal random variables. Hence,

$$f(s) = \frac{e^{-m}}{0!}\delta(s) + \cdots \frac{e^{-m}}{k!}m^k \cdot \text{normal } (kv, \sqrt{k}\,\sigma). \tag{11}$$

Components of the distribution given by equation 11 are shown in Fig. 3 *b*.

(c) Random responses of constant amplitude v of m.e.p.p.'s. Suppose that all m.e.p.p 's have a constant amplitude $A = v$, but their time of arrival is governed by the variable rate $\alpha(t)$, as shown in Fig 2. Let m.e.p.p.'s have pulse shapes given by

$$x = v \cdot h(t). \tag{12}$$

Suppose that e.p.p. is composed of two m.e.p.p.'s with times of arrival t_1 and t_2. The peak amplitude s of e.p.p. will then be

$$s = v + x.$$

That means one of the m.e.p.p.'s is added with full amplitude v, the other with the fraction x, which is a function of delay $\tau = t_1 - t_2$. As we are measuring maximum amplitude at $t = t_r$, it follows

$$x = v \cdot h(t_r + t_1 - t_2) = v \cdot h(t_r + \tau). \tag{13}$$

The probability distributions of t_1 and t_2 are equal and given by the distribution $\alpha(t)$. Hence, $\tau = t_1 + (-t_2)$, presents the sum of two random variables. In the same way as in the case of equation 4, the distribution, $\Phi_1(\tau)$ of the sum is expressed by the convolution

$$\Phi_1(\tau) = \alpha(t) * \alpha(-t). \tag{14}$$

It is not important which m.e.p.p. pulse is regarded as first. Hence, we can conclude that $\Phi_1(\tau) = \Phi_1(-\tau)$ and take into account the distribution for $|\tau|$.

$$\Phi(|\tau|) = 2 \cdot \alpha(t) * \alpha(-t). \tag{15}$$

The distributions $\alpha(t)$, $\Phi_1(\tau)$, $\Phi(|\tau|)$, and summation of two or more e.p.p.'s are illustrated in Fig. 2.

The distribution of the fraction x, used in addition, can be calculated from equations 13 and 15. We have random variable τ, with the distribution, $\Phi(\tau)$. This variable is transformed into the variable $x = x(\tau)$, equation 13, whose distribution $\rho(x)$ we want to calculate. The distribution, $\rho(x)$, will be the function of $\Phi(\tau)$ and $x(\tau)$ and can be obtained according to the theorem for transformation of the random variables (see e.g. Papoulis, 1965, p. 126).

$$\rho(x) = \frac{\Phi(|\tau_a|)}{|h'(\tau_a)|} + \cdots \frac{\Phi(|\tau_z|)}{|h'(\tau_z)|}, \tag{16}$$

where $\tau_a \cdots \tau_z$ are roots of equation 13. For the pulse shape, $h(t)$ of m.e.p.p.'s, as shown in Fig. 2, there will always be only one root, τ_a, for a given fraction, x. We now have

$$\begin{aligned} g_1(s) &= P(s) = \delta(s - v), \\ g(x) &= \rho(x), \\ g_2(s) &= \delta(s - v) * \rho(x), \\ g_k(s) &= \delta(s - v) * \rho(x) * \cdots \rho(x). \end{aligned} \tag{17}$$

From Fig. 2, we can conclude that the most probable fraction, x, will be v and that probability distribution, $\rho(x)$, will exponentially decrease as $x \to 0$. Fig. 3 *c* shows the distribution, $f(s)$, which one can obtain by inserting equation 17 into equation 1.

(*d*) *Random times, random amplitudes of m.e.p.p.'s.* We come now to the complete model in which both times of arrivals and amplitudes of m.e.p.p.'s are random variables with distribution functions $\Phi(\tau)$ and $P(A)$. The difference from the previous case is that the fraction x is now a function of $P(A)$. Knowing $\rho(x)$ for $A = v =$ constant, one can calculate the distribution $g(x)$ for the case when A is a random variable.

If A = constant = 1, the distribution of the fraction x will be $\rho(x)$, equation 16. If amplitude is A instead of 1, the distribution will have the same shape but the scale will be multiplied by A; hence, it will be

$$\frac{1}{A}\rho\left(\frac{x}{A}\right).$$

As A can have any value between $+\infty$ and $-\infty$, there will be a large number of cases producing the value x. All these cases, multiplied by the probability for a given A, and integrated, will form the probability for x:

$$g(x) = \int_A P(A)\cdot\rho\left(\frac{x}{A}\right)\cdot\frac{1}{A}\,dA. \tag{18}$$

We now have

$$\begin{aligned} g_1(s) &= P(s), \\ g_2(s) &= P(A)*g(x), \\ g_k(s) &= P(A)*g(x)\cdots g(x). \end{aligned} \tag{19}$$

If m.e.p.p.'s have pulse shapes as shown in Fig. 2, and if $P(A)$ is normal distribution, the $g(x)$ will be of similar shape as $\rho(x)$ but with rounded ends around v, where v is the mean value of m.e.p.p.'s amplitudes. This case is illustrated in Fig. 3*d*.

RESULTS

The probability distribution function $f(s)$ of e.p.p.'s amplitudes s obtained from experimental data, show peaks at 1, 2, 3, etc., times the mean amplitude of the spontaneous miniature potentials. The data in peaks are distributed according to the Poisson law with the mean quantum content m. It is shown here that the shape of the peaks is a function of the m.e.p.p.'s amplitude distribution $P(A)$, m.e.p.p.'s pulse shape $h(t)$, latency distribution $\alpha(t)$, and the mean quantum content m. The developed theory takes all of these functions into account. Through sequential use

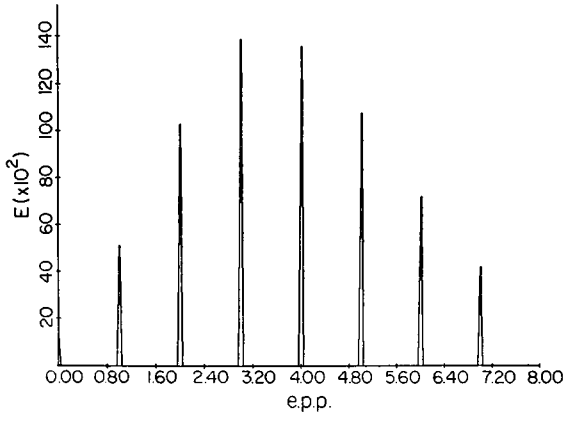

FIGURE 4 The distribution function $f(s)$ for $\nu = 1, \sigma = 0, m = 4$, no latency fluctuation. $E(\times 10^2)$ measured to nearest hundredth.

of equations 15, 16, 18, 6, and 1, one takes into account influences of $\alpha(t)$, $h(t)$, $P(A)$, and m, respectively. Next, we shall show a few applications using experimental data now available.

The latency fluctuation $\alpha(t)$ is shown in Fig. 2. The $\alpha(t)$ curve is composed on the basis of experimental data from the measurement of the time course of acetylcholine release at the neuromuscular junction in a frog muscle (Katz and Miledi, 1965). Constant delay of $\sim$0.5 msec for release rate $\alpha(t)$ is not shown because it does not influence the process.

The pulse shape $h(t)$ of m.e.p.p.'s, has exponential form. Its amplitude, rise time, and half-fall time depend on the distance from the end-plate focus, on the temperature, etc. In mammalian muscle, the rise time and the half-fall time are about one-half and one-third, respectively, of the corresponding values for the frog e.p.p. In our analysis, we shall take $h(t)$ for the frog e.p.p. (Katz and Miledi, 1965), as shown in Fig. 2.

M.e.p.p.'s amplitude distribution function $P(A)$ has been measured in many experiments. $P(A)$ can be presented with the gaussian distribution, having the mean value $\sim$0.5 mv, and coefficient of variation (i.e. standard deviation divided by the mean), between 0.1 and 0.2. The value depends largely on the experimental conditions and the noise level in the system. In our analysis, we shall take the value 0.1.

On the basis of the theory, a computer program for calculation of e.p.p.'s distribution function $f(s)$ has been written. The program[1] reads experimental data describing $\alpha(t)$, $h(t)$, $P(A)$, and m, and plots the function $f(s)$. The program can be used to describe different models for building e.p.p.'s from m.e.p.p.'s.

The simplest model takes into account only the mean quantum content m. The

[1] The program "JILL" for the study of the statistical composition of the e.p.p. is available on request from the author.

distribution function $f(s)$, for $\alpha(t) = \delta(t)$, $v = 1$, $\sigma = 0$, and $m = 4$, is shown in Fig. 4.

The next model takes into account m and m.e.p.p.'s amplitude distribution, $P(A)$. The distribution function $f(s)$, for $\alpha(t) = \delta(t)$, $v = 1$, $\sigma = 0.1$, and $m = 4$, is shown in Fig. 5. This model can also cover the cases when $P(A)$ is skewed distribution.

The next model assumes m.e.p.p.'s amplitude being constant value $A = v$, $\sigma = 0$, but takes into account the latency distribution $\alpha(t)$, and pulse shape $h(t)$, Fig. 2. Due to the delay between m.e.p.p.'s, which are summed into e.p.p., the obtained distribution is composed of the exponential peaks. The case for $m = 4$ is shown in Fig. 6.

The most complete model takes all functions into account. The distribution

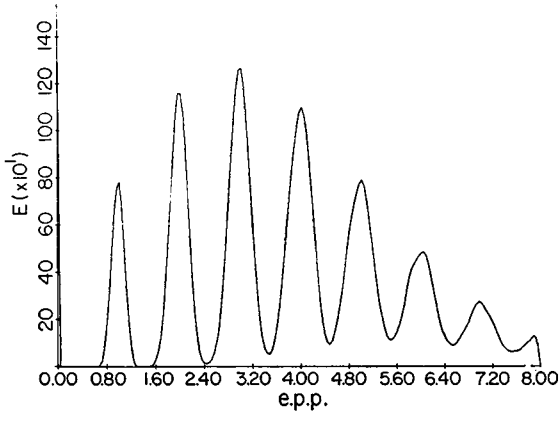

FIGURE 5 The distribution function $f(s)$ for $v = 1, \sigma = 0.1, m = 4$, no latency fluctuation. $E(\times 10^1)$ measured to nearest hundredth.

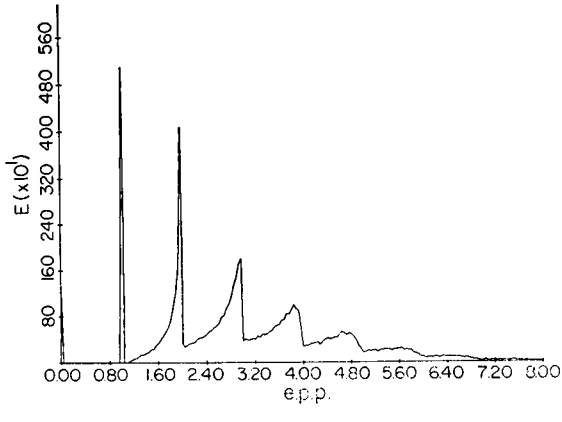

FIGURE 6 The distribution function $f(s)$ for $v = 1, \sigma = 0, m = 4, \alpha(t)$ and $h(t)$ as in Fig. 2. $E(\times 10^1)$ measured to nearest hundredth.

$f(s)$, for $\alpha(t)$ and $h(t)$, as in Fig. 2, and for $v = 1$, $\sigma = 0.1$, and $m = 4$, is shown in Fig. 7. This distribution is composed of asymmetrical peaks.

The last model is repeated in Fig. 8, for pulse shape with 50% faster fall time (mammalian e.p.p.).

One can notice the difference between distributions in Figs. 5–8. In Fig. 5, all the peaks are symmetrical and centered around the values 1, 2, 3, etc. In Fig. 6 peaks are completely asymmetrical. In Fig. 7, peaks are asymmetrical and centered around values smaller than 1, 2, 3. In Fig. 8, peaks are also asymmetrical but broader than those in Fig. 7. Only the leftmost peaks in Figs. 7 and 8 are identical (e.p.p. composed of only one m.e.p.p.; hence, the latency fluctuation cannot influence this peak).

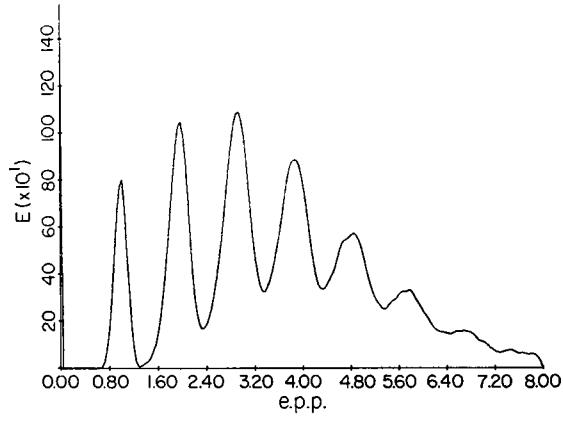

FIGURE 7 The distribution function $f(s)$ for $v = 1, \sigma = 0.1, m = 4, \alpha(t)$ and $h(t)$ as in Fig. 2. $E(\times 10^1)$ measured to nearest hundredth.

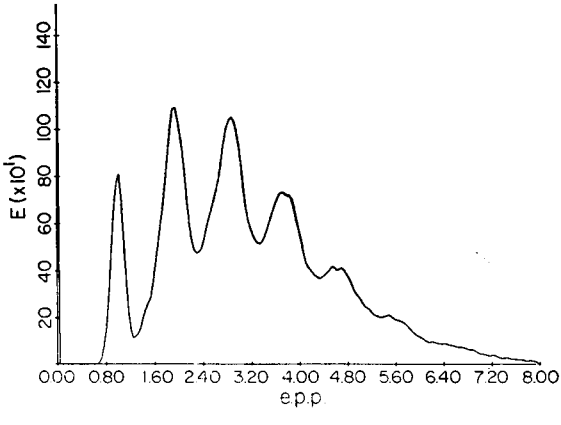

FIGURE 8 The distribution function $f(s)$ for $v = 1, \sigma = 0.1, m = 4, \alpha(t)$ as in Fig. 2, $h(t)$ 50% faster fall time. $E(\times 10^1)$ measured to nearest hundredth.

The relative intensities of the peaks depend on the mean quantal content m and on the standard deviation of m.e.p.p.'s amplitudes. For values of m less than 3, the distribution will have progressively decreasing peaks as in Fig. 1 ($m \sim 2.5$). For values of m larger than 3, the distribution will have the highest peaks for the amplitudes $m - 1$ and $m - 2$, as in Figs. 7 and 8 ($m = 4$).

CONCLUSION

From the results shown, one can draw a few conclusions.

(*a*) The distribution function $f(s)$ of the e.p.p.'s amplitude is only approximately composed from gaussian peaks. Detailed analysis shows that the peaks are asymmetrical due to the influence of the latency fluctuations.

(*b*) The mean number of quantal components released by an impulse m may be obtained in the first approximation by dividing the mean amplitude of a series of e.p.p.'s recorded from the fiber by the mean amplitude of the m.e.p.p.'s. This simple rule can only be used provided that m is small and the response does not exceed a few millivolts in size. For larger responses, a correction must be applied because miniature potentials do not add linearly beyond a limited range (Martin, 1955). Another correction of m is necessary which will take into account the effect of the latency fluctuations. Instead of the mean amplitude of m.e.p.p.'s, one should calculate m using the mean value of the function $g(x)$, equations 16 and 18.

(*c*) For transmission study at low temperature, one can expect the latency fluctuations to be more pronounced and to make stronger influence on the e.p.p.'s distribution. Special cases are skewed distributions of m.e.p.p.'s. Using equation 9, one can calculate e.p.p.'s distribution for such cases.

(*d*) To make a further study of the statistical composition of the e.p.p., new measurements should be necessary concentrating on the noise reduction, correlation investigation and multichannel pulse data analysis (see e.g. Souček, 1969 *a,b*). Results can be used to develop more detailed models of the quantal process of the transmitter release.

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