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QUANTAL COMPONENTS OF THE END-PLATE POTENTIAL

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In this paper a further study is made of the spontaneous synaptic potentials in frog muscle (Fatt & Katz, 1952*a*), and their relation to the end-plate response. It has been suggested that the end-plate potential (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 e.p.p.'s'. The latter, therefore, could be regarded as the least unit, or the 'quantum', of end-plate response. A convenient picture of how hundreds of such quanta, each capable of producing a miniature potential of 0·5–1·0 mV, can build up an e.p.p. of, say, 70–80 mV is provided by the hypothesis that separate parcels of acetylcholine (ACh), released from discrete spots of the nerve endings, short-circuit the muscle membrane. The unit changes of membrane conductance produced at many parallel spots summate and lead to an intense depolarization of the muscle fibre.

Although this is a plausible view, there is no direct proof that the normal e.p.p. is made up in this quantal fashion. The evidence comes from experiments in which the 'quantum content' of the e.p.p. had been reduced to a small number by lowering the external calcium concentration (Fatt & Katz, 1952*a*). It was then found that the size of the end-plate response approached that of the spontaneous potential and at the same time exhibited large random fluctuations, apparently involving steps of unit size. Similar observations were made by Castillo & Engbaek (1954) on muscles treated with Mg-rich solutions. The statistical behaviour of the end-plate response under these conditions has been investigated in more detail and subjected to a quantitative analysis.

METHODS

The technique for intracellular recording of e.p.p.'s and miniature potentials has been described in earlier papers (Fatt & Katz, 1951, 1952*a*; Castillo & Katz, 1954). The m. ext. l. dig. IV of English frogs was used, immersed in an isotonic solution containing concentrations of $CaCl_2$ and $MgCl_2$ adjusted so as to reduce the response to any desired level. In most experiments prostigmine (10^{-6}, w/v) was added to increase the amplitude of the potentials (without altering their 'quantum content'). The usual procedure was to locate a suitable spot with the internal electrode and record

spontaneous potentials on moving film. Then, a large number of end-plate responses to single or pairs of nerve volleys were recorded, using a swept time-base and one or a few seconds interval between records. Finally, another series of spontaneous potentials was recorded before the microelectrode was withdrawn from the fibre.

The amplitudes of the potentials were measured and their distribution displayed in a histogram. With low Ca and high Mg concentrations, all-or-none fluctuations are observed in successive records, with frequent total failures of e.p.p. response. Special importance was attached to the counting of 'failures' and 'successes', as their proportions provided a simple and decisive test of our hypothesis. A precaution which had to be taken was to guard against intermittent failures of response due to other causes, e.g. inadequate stimulation or nerve damage leading to block. The first source of trouble was avoided by using a strong shock, the second source could be recognized without much difficulty, because nerve block, if it occurred at all, developed in a rapidly progressive manner and was unrelated to the size of the initial end-plate response.

In several muscle fibres there was evidence of a remote, second, motor nerve supply (cf. Katz & Kuffler, 1941) producing small and slow miniature potentials and e.p.p. response. These were of discrete shape and could be discarded without ambiguity, when counting responses and measuring amplitudes.

The ext. l. dig. IV contains some muscle fibres of the 'slow system' supplied by small motor axons (Kuffler & Vaughan Williams, 1953; Katz, 1949). As most experiments were made below the level of propagated spikes, the question arises whether we may not sometimes have been recording from 'slow' muscle fibres. This is unlikely because the characteristics of the response were those of the e.p.p.'s of 'twitch fibres' (sharp localization and low threshold whenever tested, high resting potential, short latency, monophasic e.p.p. response). Spontaneous discharge of miniature potentials had previously been shown to occur at end-plates of ordinary 'twitch fibres' (Fatt & Katz, 1952*a*).

RESULTS

When a muscle was soaked in a solution containing approximately 10 mM-$MgCl_2$, transmission became blocked and subthreshold e.p.p.'s could be recorded at individual junctions. A characteristic feature of these responses was their random fluctuation in successive records. This is illustrated in Fig. 1 where twelve responses, together with some spontaneous miniature potentials, are shown. If the response was further reduced, by increasing Mg or lowering Ca concentrations, the amplitude fluctuations became even more pronounced and were found to be of discontinuous character. In the experiment of Fig. 2, for instance, the majority of records showed no response at all. On the average only about one out of seven nerve impulses elicited an e.p.p. whose size was of the same order of magnitude as the spontaneous potentials.

This behaviour is characteristic of block by high Mg and low Ca, and very different from curare-block. With increasing doses of curarine the e.p.p., at individual junctions, is progressively reduced in size and may eventually become undetectable, but we have never found the response to be abolished, or to fluctuate, in the quantal manner shown in Fig. 2.

If one proceeds to add Mg or withdraw Ca, a practical limit is reached when the e.p.p. response becomes too infrequent to be distinguished from a spontaneous discharge. There are no differences in amplitude which would enable one to discriminate between the two forms of activity; the distinction

depends entirely on the constant latency of the response and random timing of the spontaneous discharges. In a normal frog muscle, at 20° C, the latency of the e.p.p. varies only within a fraction of a millisecond, but in the present experiments we have accepted 1–2 msec as the maximum latency fluctuation, and disallowed as 'response' any potentials which arose outside these limits. In practice, unless the frequency of spontaneous firing was high and the

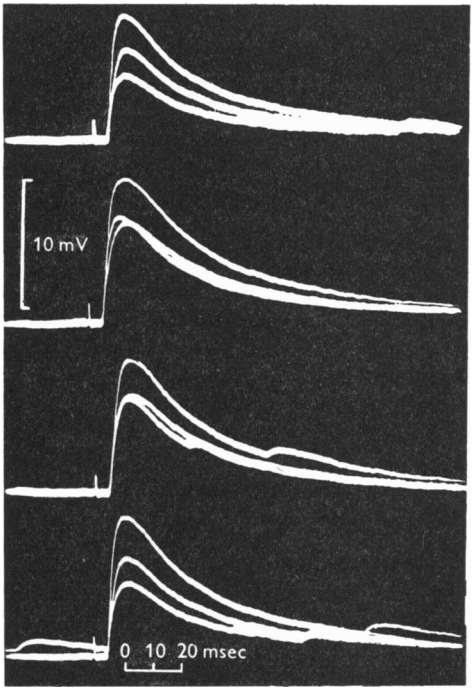


Fig. 1. Fluctuation of e.p.p. response at a single nerve-muscle junction, treated with 10 mM-Mg (Ca concentration was normal: 1·8 mM; prostigmine 10^{-6}). Intracellular recording. In each record, three superimposed responses are seen. Note scattered spontaneous miniature potentials.

frequency of responding very low, there was little chance of confusing a spontaneous potential with an e.p.p.-response: for example, in Fig. 2 (latency of five 'accepted responses' being constant within 1 msec; spontaneous firing rate 2·2 per sec) the chances of one of the 'accepted responses' being 'spontaneous' are about 5%, and the chances of more than one arising spontaneously are quite negligible.

Most experiments were made at an intermediate level of blocking when the proportion of failures at individual end-plates was of the order of 50%. The remaining responses were scattered in amplitude over a wide range, as illustrated in Fig. 3. (Responses to pairs of nerve impulses are shown in this

figure.) Many e.p.p.'s fall evidently within the range of sizes of the spontaneous potentials. Others are larger and probably represent multiple units of response. It is interesting that the large e.p.p.'s occasionally show a just noticeable inflexion on their rising phase (e.g. Fig. 3, record C_1) indicative of their composite nature and of imperfect synchronization of the contributing units.

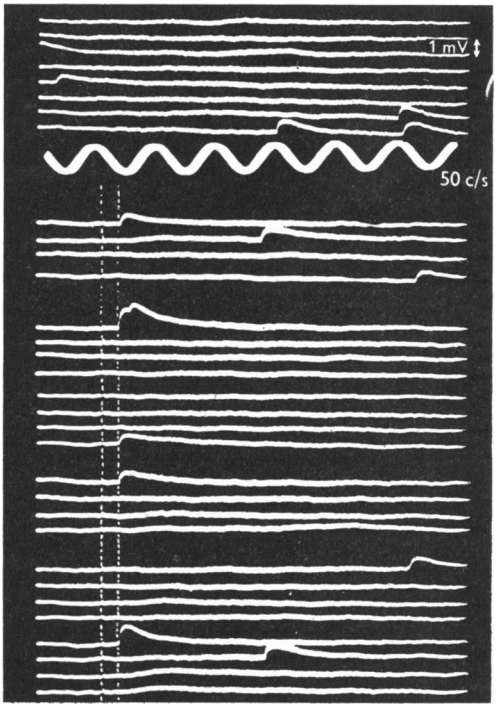


Fig. 2. This muscle was treated with reduced Ca (0·9 mM) and 14 mM-Mg concentration. The top part shows a few spontaneous potentials (traces separated by 1 mV steps). The lower part (below the 50 c/s time signal) shows responses to single nerve impulses. Stimulus artifact and response latency are indicated by a pair of dotted vertical lines. The proportion of failures was very high: there are only five responses to twenty-four impulses.

The experiments of Figs. 1–3, made at different levels of neuromuscular block, have one feature in common, namely a wide fluctuation in e.p.p. amplitudes. In Figs. 4 and 5, the distribution of amplitudes in two experiments is shown, both of spontaneous potentials and response. It is clear that these results cannot be analysed, nor even satisfactorily described, without a statistical treatment.

Suppose we have, at each nerve-muscle junction, a population of n units (cf. Fatt & Katz, 1952*a*, 1953) capable of responding to a nerve impulse. Suppose, further, that the average probability of responding is $\overline{p}$ (the chances p

may differ greatly for the individual constituents, but are supposed to remain constant during the experiment) then the mean number of units responding to one impulse is $m=n\bar{p}$. Under normal conditions, $\bar{p}$ may be assumed to be relatively large, that is a fairly large part of the synaptic population responds to an impulse. However, as we reduce the Ca and increase the Mg concentration, the chances of responding are diminished and we observe mostly

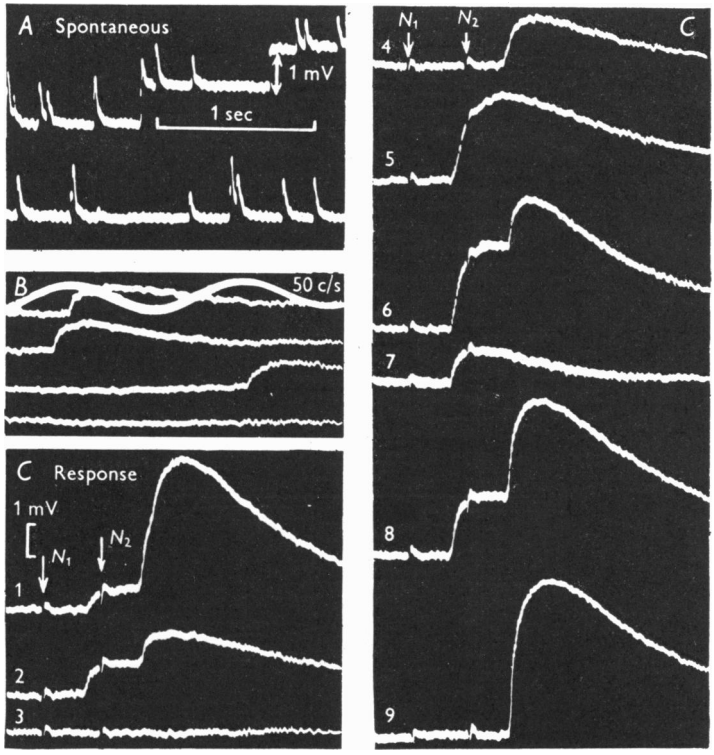


Fig. 3. Muscle was treated with a solution containing 0·45 mM-Ca and 6 mM-Mg. Intracellular recording from single junction. *A* and *B*: spontaneous miniature e.p.p.'s. *C*: examples of responses to paired nerve impulses. Timing of stimuli N_1 and N_2 is indicated by arrows. Failure of response to N_1 in C_4 and C_9, failure to N_2 in C_5 and C_7, double failure in C_3. 50 c/s time signal applies to *B* and *C*. *A* was recorded on slow time base and shows two calibration steps of 1 mV.

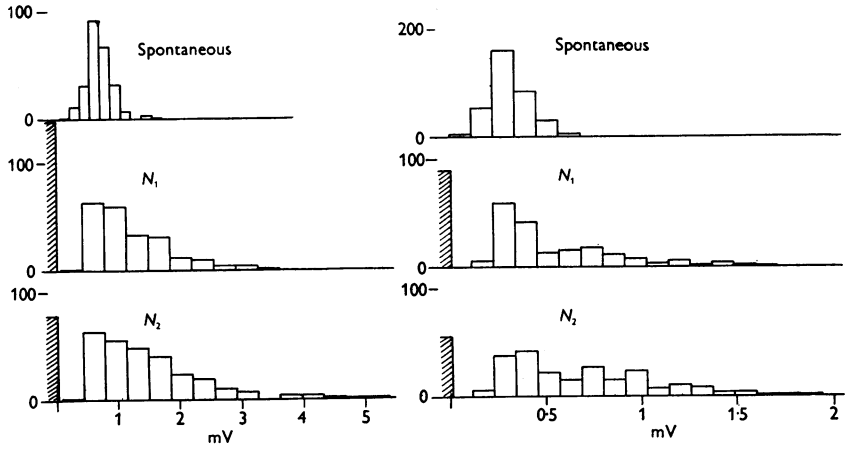
complete failures with an occasional response of one or two units. Under these conditions, when p is very small, the number of units x which make up the e.p.p. in a large series of observations should be distributed in the characteristic manner described by Poisson's law (their relative frequencies being given by $\exp(-m)\, m^x/x!$).

To test the applicability of Poisson's law may seem difficult, because all we can do is measure amplitudes of supposedly composite e.p.p.'s; we cannot

count the components directly. The task is, however, made easier because the presence of spontaneous activity gives us an independent measure of unit size.

We can obtain the value of m, i.e. the mean number of units responding to one impulse, in two ways: first, from the relation

$$m = \frac{\text{mean amplitude of e.p.p. response}}{\text{mean amplitude of spontaneous potentials}}. \tag{1}$$



Figs. 4 and 5. Histograms from two end-plates, showing distribution of amplitudes of spontaneous miniature potentials and of the responses to pairs of nerve impulses (7 msec interval between N_1 and N_2). *Failures* are not represented as a 'class', but their number is indicated by the height of the shaded columns.

Equation (1) is a simple re-statement of our hypothesis, namely that the e.p.p. is made up of units of the same size (though not necessarily composed of the same individuals) as the spontaneous miniature potentials. Equation (1) depends on the assumption that there is linear summation of the miniature components of the e.p.p.: this is justified provided the amplitude of the e.p.p. is only a few per cent of the resting potential (cf. Fatt & Katz, 1951), but equation (1) fails to apply to larger responses.

Secondly, we can use the first term of the Poisson series ($\exp(-m)$, for $x=0$) which gives the proportion of failures. Hence

$$m = \log_e \frac{\text{number of nerve impulses}}{\text{number of failures of e.p.p. response}}. \tag{2}$$

Combining (1) and (2) we obtain

$$\frac{\text{mean amplitude of response}}{\text{mean amplitude of spontaneous potentials}} = \log_e \frac{\text{number of impulses}}{\text{number of e.p.p. failures}}. \tag{3}$$

Equation (3) provides a useful test of our hypothesis and depends only on measurements of mean amplitudes and counting of 'failure' and 'success' of e.p.p. response. The results of several experiments in which this test has been applied are shown in Table 1 and Fig. 6. The agreement between the two

TABLE 1. In the last two columns the validity of equation (3) is tested in ten experiments. They include four experiments in which responses to pairs of impulses have been utilized (N_1–N_2 intervals 3·5–11 msec). It will be seen that the value of m (i.e. A/B or $\log_e C/D$) is larger for N_2 than for N_1, an effect which is discussed in the following paper.

Date		Mean response (mV) (A)	Mean spont. potential (mV) (B)	No. of impulses (C)	No. of failures (D)	A/B	$\log_e C/D$
2. vi. 51		0·495	0·875	328	188	0·57	0·56
23. i. 53, A	N_1	0·334	0·46	289	113	0·73	0·94
	N_2	0·588			76	1·28	1·33
23. i. 53, B	N_1	0·358	0·305	280	89	1·17	1·15
	N_2	0·528			56	1·73	1·61
28. i. 53	N_1	0·727	0·72	357	138	1·01	0·95
	N_2	1·14			78	1·58	1·52
4. ii. 53	N_1	0·495	0·335	319	84	1·48	1·33
	N_2	0·905			27	2·7	2·47
24. ii. 53		0·089	0·565	118	99	0·16	0·18

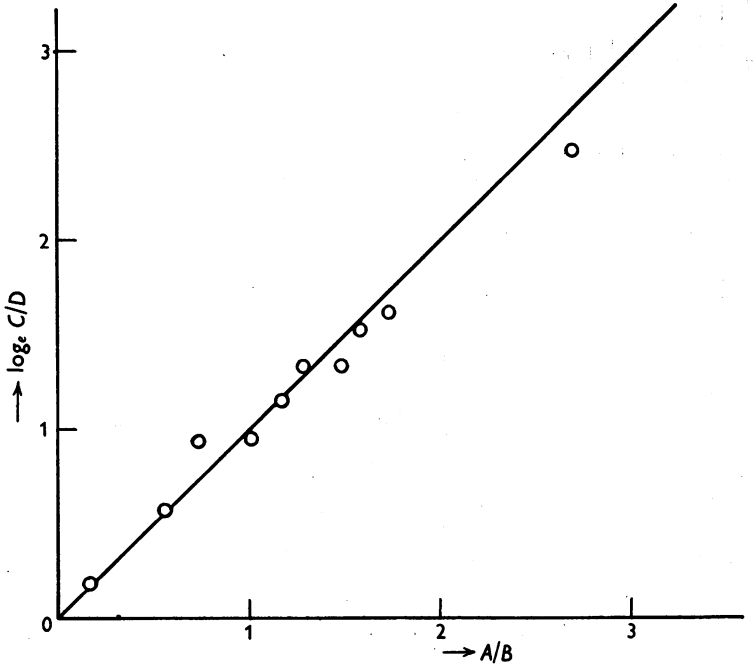


Fig. 6. The results of ten experiments summarized in Table 1 have been plotted, showing the consistency of the two methods of determining the value of m (equations (1) and (2)). Ordinate: $\log_e \frac{\text{number of impulses}}{\text{number of e.p.p. failures}}$. Abscissa: $\frac{\text{mean e.p.p. response}}{\text{mean amplitude of spontaneous potentials}}$. The line corresponds to equality of these two estimates of m.

determinations of m, corresponding to the right and left sides of equation (3), is very satisfactory and may be regarded as a strong support of our initial hypothesis.

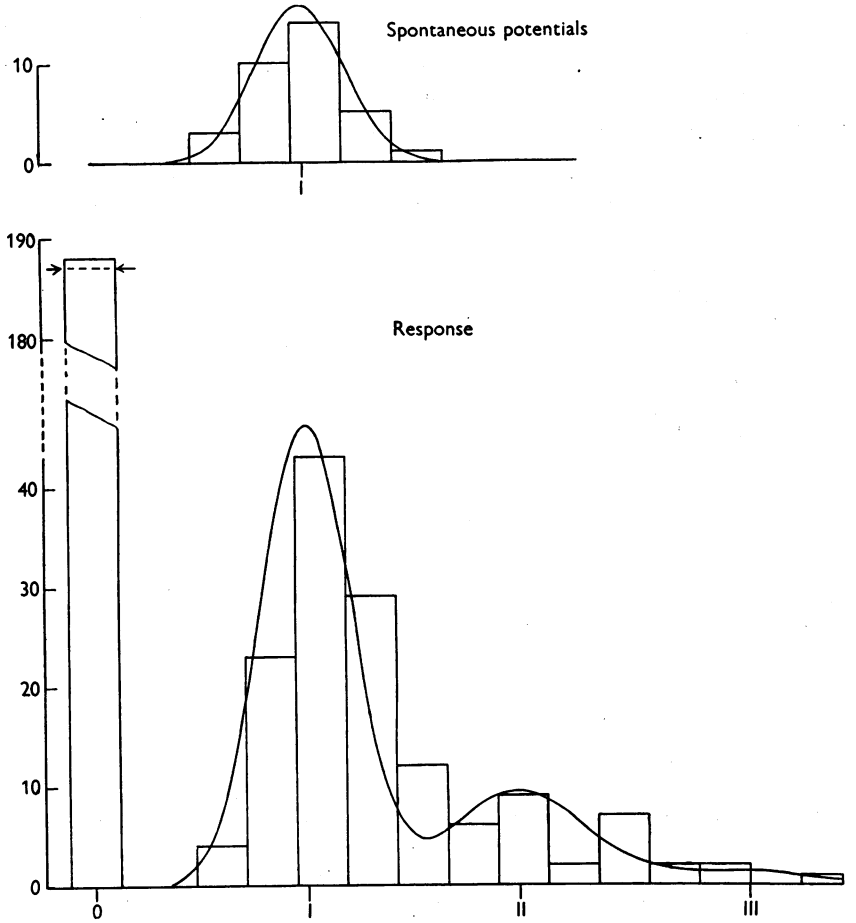


Fig. 7. Histogram showing distribution of amplitudes of spontaneous miniature potentials and end-plate responses at a Ca-deficient junction (experiment of Fatt & Katz, 1952*a*, pp. 119–120). In the lower part, the continuous curve has been calculated on the hypothesis that the responses are built up statistically of units whose mean size and amplitude distribution are identical with those of the spontaneous potentials (see text). Expected number of failures shown by arrows. Abscissae: scale units = mean amplitude of spontaneous potentials (0·875 mV).

The experiment of Fig. 7, the results of which were reported by Fatt & Katz (1952*a*), has been analysed more fully. The value of m was first determined by equation (1), and the expected numbers of the Poisson series were calculated. For $x=0$ (failure of response), there was excellent agreement between calcu-

lated and observed values, but for the terms $x > 0$ account had to be taken of the scatter of amplitudes of the 'unitary' spontaneous potentials. This was done by (*a*) fitting a Gaussian curve to the spontaneous potentials, and (*b*) using x times the mean and variance of this curve in distributing the Poisson classes. The resulting theoretical distribution of e.p.p. amplitudes is shown by the continuous curve in the lower part of Fig. 7. Although the fit with the observed histogram is not accurate, the general agreement is good considering that except for a single scaling factor (the total number of e.p.p.'s) the

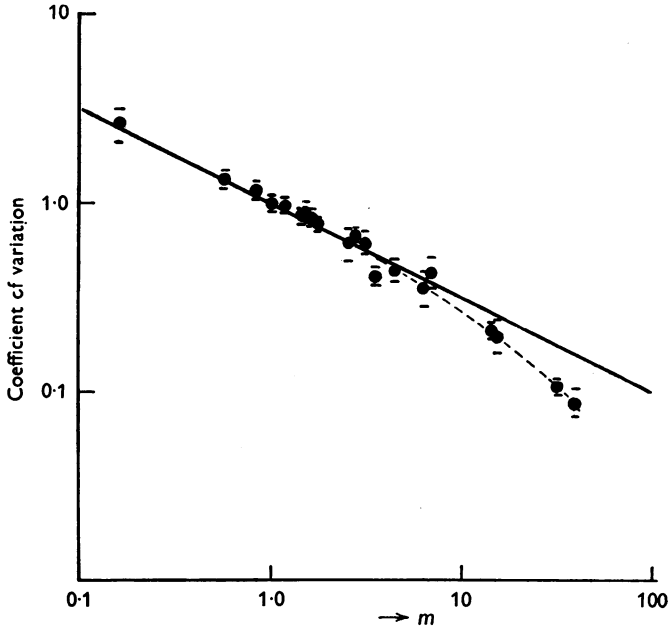


Fig. 8. Relation between coefficient of variation and mean amplitude of e.p.p. in twenty-one experiments. Logarithmic scales. Abscissa: mean e.p.p., divided by mean spontaneous potential (i.e. nominal value of m). Ordinate: standard deviation of e.p.p., divided by mean (i.e. 'coefficient of variation' of e.p.p.). E.p.p. amplitudes had been grouped for this purpose in 'unit classes' (i.e. with class centres at $n \times$ mean spontaneous potential). Bars have been placed at ± 2 S.E. of the 'coefficient of variation'. Full line shows theoretical relation for Poisson-distributions.

constants chosen in calculating the curve had been determined independently. The main discrepancies vanish if the mean size of the unit response is taken to be 7% larger than the mean spontaneous potential, a difference which is probably within limits of experimental error.

In other experiments the e.p.p. amplitudes were grouped more coarsely into classes of unit-width, and a χ^2 test was applied; also, the coefficient of variation of e.p.p. amplitudes (grouped in such unit classes) was determined and compared with the expected coefficients $m^{-0.5}$ (Fig. 8). These tests were less

accurate than the preceding analysis, but the results agreed with the view that the responding units are distributed in Poisson-fashion provided the quantum content of the e.p.p. is small (< 3). When the tests were extended to larger e.p.p.'s (m exceeding 10), there was a consistent discrepancy, the observed fluctuation of e.p.p. amplitudes covering a smaller range than expected (see Figs. 8 and 9).

DISCUSSION

The most interesting evidence is that shown in Table 1 and Fig. 6 for small values of m. The agreement between the two determinations of m can hardly be fortuitous and supports the view that the spontaneous miniature potential is the least 'quantum of action' at the nerve-muscle junction, the e.p.p. being built up statistically of such quanta. Furthermore, one may conclude that at this reduced level of m, the statistical chances of any one unit responding to a single impulse are very low, and in successive records the responses represent different members of a large, mostly inactive, population.

It is tempting to speculate what the precise probability of the unit response may be. For this, it is not sufficient to know only the value of m; we also require information of the total number of available units n. Moreover, it does not follow from the results that all units have the same chance of responding; a Poisson distribution would be obtained even from a non-uniform population, provided only the probabilities of responding are small and constant for each individual member (Kendall, 1948). If the whole synaptic population consisted of, say, 500 units, and m is unity, then the average chance of any unit responding to one impulse would be 1/500, but individual probabilities may be considerably higher for some and much smaller for many other members of the population.

What happens under more normal conditions when we raise the Ca and lower the Mg concentration? The value of m becomes large and the statistical analysis unsatisfactory. It is clear, however, that the response fluctuates much less than predicted from our equations (Fig. 9). Now suppose the size of the population n remains constant, then the increase of m would be due to an increased probability p. If the population is uniform, the distribution of responses would change from a Poisson to a binomial form. Associated with this one may expect a reduction in statistical spread, for the coefficient of variation for a Poisson series is $\sqrt{(1/m)}$, while that of a binomial distribution is only $\sqrt{\left(\frac{1}{m}-\frac{1}{n}\right)}$. Closer examination, however, shows that this argument is insufficient to account for the observed divergences. We can set a lower limit to the value of n: the normal e.p.p. is about 100 times larger than a miniature potential and must be composed of an even greater number of units because unit increments of the e.p.p. would diminish at high levels of depolarization (cf. Fatt & Katz, 1951). There is also reason to believe that the normal e.p.p.

does not involve the whole population, so that $n=200$ is a conservative estimate. With $m=32$, the coefficient of variation would be $\sqrt{(\frac{1}{32}-\frac{1}{200})}=0{\cdot}162$, compared with 0·177 in a Poisson distribution (when m/n is very small). The observed coefficient, however, is about $0{\cdot}11\pm0{\cdot}005$, and a significant discrepancy of this kind remained for all experiments in which m was greater than 10.

There are two other factors which are more likely to provide an explanation. One factor has already been mentioned, viz. a failure of linear summation of miniature potentials, when m becomes large and the amplitude of the total e.p.p. an appreciable fraction of the resting potential. Application

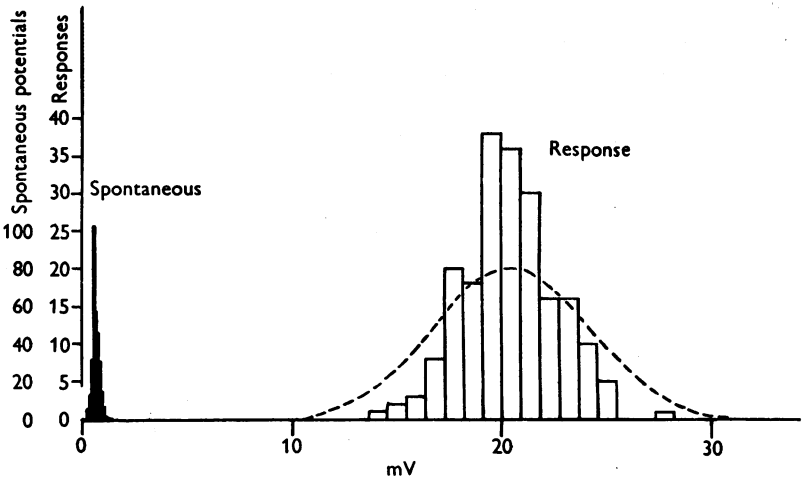


Fig. 9. Histogram from an experiment with large e.p.p. Nominal value of m (using equation (1)) is 32. Dotted curve: expected distribution of e.p.p.'s (modified Gaussian curve allowing for scattered unit size: mean = 20·4 mV), $\sigma=3{\cdot}7$ mV. Note large discrepancy between observed and expected distribution.

of equation (1) and of the superposition theorem may lead to serious error if the e.p.p. response exceeds a small fraction (5%) of the resting potential. Suppose each 'transmitter unit' produces a fixed leakage conductance ΔG across the end-plate membrane, then the increment of potential ΔP which it contributes to the e.p.p. becomes less the greater the existing leakage and the lower the membrane potential (cf. Fatt & Katz, 1951). This must have an important effect on the observed coefficient of variation, because (*a*) the actual number m would be greater than that calculated from equation (1), and (*b*) the scale of the amplitude fluctuations would be reduced in proportion to ΔP. We have made only a rough estimate of this effect, but it seems that it may account for a large part, if not the whole, of the observed discrepancy.

The other factor which may be involved is that different members of the population may *not* have the same chances of success, and that for large

values of m some individual units have a high probability and respond almost every time, while others have a low probability and contribute to the e.p.p. only occasionally. The presence of some units which respond regularly is bound to diminish the statistical fluctuation of the e.p.p. In general, the coefficient of variation for this case is less than that expected for a binomial distribution (see Table 2).

TABLE 2. Coefficients of variation for different distributions

(From Kendall, 1948; the coefficient of variation is expressed here as a simple fraction, instead of per cent.)

Poisson ($p \ll 1$)	Binomial (var $p = 0$)	'Non-uniform population'
$\sqrt{\left(\frac{1}{m}\right)}$	$\sqrt{\left(\frac{1}{m} - \frac{1}{n}\right)}$	$\sqrt{\left(\frac{1}{m}\left(1 - \frac{\text{var } p}{\bar{p}}\right) - \frac{1}{n}\right)}$

n = total number of units available at a single junction.
m = mean number of units responding to one impulse.
$\bar{p} = m/n$ = average probability of response (per unit per impulse).
var p = variance of individual probabilities (p being assumed to vary among responding units, but not during successive impulses).

This last factor should not be confused with the case in which probabilities of response vary during the set of observations, e.g. if the value of m suffered a progressive change. In this case the standard deviation of the e.p.p. amplitude would become greater, not less. A small effect of this kind was present in some experiments and could be checked by dividing the observations into groups. The drift of the mean value, however, was not large enough to affect the result seriously.

CONCLUSIONS

The following picture emerges from the present study: transmission at a nerve-muscle junction takes place in all-or-none 'quanta' whose sizes are indicated by the spontaneously occurring miniature discharges. The number of quantal units responding to a nerve impulse fluctuates in a random manner and can be predicted only in statistical terms. The average 'quantum content' of the e.p.p. depends on the probability of response of the individual units, and this varies with the external Ca and Mg concentration (for a more detailed hypothesis, see Castillo & Katz, 1954). It is possible that some synaptic units respond more readily than others, but with a sufficiently high Mg and low Ca level the chances of excitation of all units are so small that a Poisson distribution is obtained.

Under more normal conditions, the e.p.p. is large and the statistical fluctuation small. While the evidence for the quantal composition of low-level e.p.p.'s ($m < 5$) seems conclusive, inferences about the normal behaviour are indirect and can only be made by extrapolating into a range in which the present statistical analysis can give no useful information. There are, however, good reasons for supposing that the normal e.p.p. is built up of a large number of units of the same kind as described here, furthermore that even the normal e.p.p. involves only a fraction of the total synaptic population, the average probability of response apparently being less than unity. This suggestion is

based on the finding that the size of the e.p.p. can be increased from nil to well above the 'normal-Ringer' amplitude by raising the Ca concentration, *without* increasing the size of the spontaneous miniature e.p.p. (Fatt & Katz, 1952*a*, *b*; Castillo & Stark, 1952). If one accepts the present results as showing that the miniature e.p.p. is the basic unit of response, then the effect of Ca must be to raise the quantum content m of the e.p.p., either by increasing the size of the population n or its probability of responding $\bar{p}$. We have assumed in our argument that a change of probability, rather than population size, is involved, though the formal distinction between these two modes of action is not very profitable until more is known about the nature of the molecular reaction whose probability we are considering.

SUMMARY

1. The relation between response and spontaneous activity at a single nerve-muscle junction has been studied.

2. By increasing Mg and lowering Ca concentration, the amplitude of the e.p.p. can be reduced to that of a spontaneous 'miniature potential'. At the same time, a large random fluctuation of successive e.p.p. amplitudes is observed.

3. Statistical analysis indicates that the e.p.p. is built up of small all-or-none quanta which are identical in size and shape with the spontaneously occurring miniature potentials.

4. When the average 'quantum content' (m) of the e.p.p. is small ($m < 3$), its amplitude fluctuates in a manner predictable by Poisson's law. At higher levels ($m > 10$), deviations occur which may be due to a reduction in the 'unit-increment' of the e.p.p., or to variation in the probability of response among different synaptic units.

5. The statistical behaviour of the normal nerve-muscle junction and the influence of Ca and Mg ions are discussed.

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