

SPONTANEOUS FLUCTUATIONS OF EXCITABILITY IN THE MUSCLE SPINDLE OF THE FROG

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The discharge of impulses from the muscle spindle of the frog, when subjected to a constant mechanical stimulus (stretch), may show great irregularity. This is most noticeable when the muscle is near its resting length. The mean rate of impulses is then very low, and successive intervals appear to vary in a random manner. As the muscle is stretched, the rate of afferent discharge increases and irregularities become less obvious (Matthews, 1931).

A possible explanation of the random discharge at low frequency has been suggested by Katz (1950*a, b*), on the basis of observations on single sensory nerve endings. Depolarization of the terminal branches of an axon in a muscle spindle was found to be responsible for the initiation of sensory impulses, the rate of the discharges depending upon the intensity of the local potential change. Fluctuations of the level of depolarization would therefore cause irregularity of the intervals between successive impulses. Such fluctuations of potential might be due directly to thermal agitation of ions in the nerve terminals, or they might be produced indirectly by molecular agitation in the mechanical receptor substance.

At present, little is known about the mechanical receptor system, but the effects of thermal agitation in nerve endings can be subjected to a tentative theoretical analysis (see Fatt & Katz, 1952). Thus, the finer the diameter of a fibre, the larger will be the amplitude of 'voltage noise' at the terminal. For example, for an extremely fine ending, 0.1μ in diameter, a voltage fluctuation of the order of 0.5 mV r.m.s. has been estimated.

The present experiments were planned to investigate whether this simple source of physical random disturbance could explain the observed fluctuations of impulse discharge. The irregularity was measured over a large range of frequencies of firing, applying various amounts of stretch to the muscle.

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Therefore, primarily the result gives quantitative information about impulse irregularity in a sensory axon. In addition, the result has some bearing on the nature of the underlying cause of irregularity.

METHODS

Preparation. The experiments were performed on M. extensor long. dig. IV of the frog, at room temperature, a preparation described in detail by Katz (1950*a*). After dissection of the muscle and its nerve, the nerve was progressively cut down at its entry into the muscle until only a single sensory axon remained, occasionally together with a motor axon. The muscle was mounted in paraffin oil, being held at one end with a rigid pair of forceps, which could be moved by a micrometer screw, and, at the other, by a lever, damped by a viscous resistance, in series with a magnetic relay. The micrometer was used for slow stretch of the muscle, the lever for rapid stretch. The preparation was kept in a metal box, placed on a thick rubber pad, in order to avoid electrical and mechanical disturbances.

Recording. Action potentials in the isolated sensory axon were recorded by Ag-AgCl-agar electrodes, one on the muscle tendon and the other on the main nerve trunk. The electrical recording equipment consisted of a cathode-follower input stage of low grid current, a resistance-capacity coupled push-pull amplifier with a 1.5 sec time constant, a double-beam cathode-ray oscillograph and a loud speaker. With stationary oscillograph beams the action potentials and a time calibration of 50 c/s were photographed on recording paper, moving at a fixed speed which could be varied from 3.3 to 56 cm/sec.

Procedure. A preparation was dissected, left for half an hour in Ringer's solution and then mounted in paraffin oil. The muscle was stretched or released to different lengths above or below its 'resting' length, as measured *in situ*, in order to produce afferent firing of different frequencies. After each stretch a series of impulses, usually several hundred, was photographed. Slow stretch sufficed to produce low firing frequencies but for the highest frequencies (100-250 imp/sec) rapid stretch up to of 25% of the 'resting' length had to be used. The speed of the recording paper was adjusted for each new frequency so as to give intervals at least 3 mm, but usually 5-10 mm, in length between the recorded spikes.

Measurement. The intervals between successive spike potentials on the recording paper were measured with a travelling microscope, graduated to 0.01 mm. The paper speed was calculated from the calibrating 50 c/s, and irregularities in speed determined.

Solutions. The Ringer solution contained 102 mm-NaCl, 1.0 mm-KCl, 0.9 mm-CaCl₂ and 1.2 mm-NaHCO₃.

RESULTS

Qualitative observations

In the course of the present investigations, we repeated and confirmed the observations made by Katz (1950*a, b*) on the form of the action potential of an afferent impulse from the muscle spindle, on the occurrence of brief prepotentials with or without a propagated impulse and on the depolarization of afferent nerve endings produced by muscle stretch.

When a muscle was adjusted to its *in situ* length, the isolated sensory axon usually fired irregularly at frequencies less than 10 imp/sec. If the muscle was considerably shortened below this length most preparations, but not all, were completely silenced for the period of observation.

When the muscle was stretched beyond its resting length, the discharge rate increased and apparently became more regular, in the manner described by

Matthews (1931). To determine residual random fluctuations it was necessary to apply statistical tests. In doing so some experiments had to be rejected because of the following complications.

In a few preparations, short bursts of impulses at a high frequency appeared at irregular intervals between periods of 'normal' activity. No correlation of these bursts with mechanical disturbances could be observed. A similar phenomenon was observed by Matthews (1931) in muscles kept in a solution of 0.75% sodium chloride. In our experiments it was taken to be a sign of abnormality and an adequate reason for discarding the preparation.

In some preparations, when the muscle was subjected to a rapid, large stretch to produce a high firing frequency, one or several impulses occasionally dropped out of an otherwise regular series of impulses. The same phenomenon had been observed earlier by Matthews (1931) and Katz (1950*b*). All recordings showing such blockings were discarded and if blocking had already occurred at fairly low firing frequencies the preparation was discarded completely.

Adaptation, that is a gradual fall of discharge rate at constant muscle lengths, became more and more apparent as a muscle was stretched by increasing amounts, as was described by Matthews (1931) (see Fig. 1 A). In fact, adaptation was one factor limiting the range within which the irregularity of impulse rate could be statistically calculated in a satisfactory manner.

Quantitative observations

Statistical computation of irregularity of impulse discharge. A single recording consisted of several hundred impulses, photographed with the muscle at constant length. The intervals between twenty-one consecutive impulses were pooled, each separate recording yielding about ten such pools. Each experiment consisted of a number of recordings, usually five to ten, covering as large a range of firing rates as possible. After a careful selection had been made to avoid 'abnormal' preparations, on criteria described above, eleven experiments with altogether 590 pools (11,800 intervals) were left for statistical treatment. For each pool the mean interval and the standard deviation (s.d.) were determined (in msec). This treatment gave 590 pairs of mean intervals and their s.d., the latter expressing the irregularity of impulse discharge in terms of intervals.

The statistical procedure was selected after careful consideration of several alternative methods. The statistical treatment was based on the use of intervals (in msec) between impulses and not their reciprocal values (rates in imp/sec). The reason for this was that the distribution of intervals about their means was symmetrical over a large range of mean intervals, from 150 to 6 msec (see Fig. 2), while for their reciprocal values (rates) the distribution was asymmetrical over a large range. For instance, Fig. 1 B shows the disparity between the distribution curves of rates and intervals at a mean rate of

11 imp/sec (corresponding to a mean interval of 107 msec). Within a range where the distribution curves are changing in skewness, the s.d. cannot be regarded as a satisfactory measure of irregularity for quantitative comparison.

The number of intervals used for each pool was twenty. The reason for not taking a larger number, to increase the fidelity of the s.d. of a pool, was the presence of adaptation at high impulse rates. Adaptation produced a continuous change of mean interval during a record (see Fig. 1 A). This could

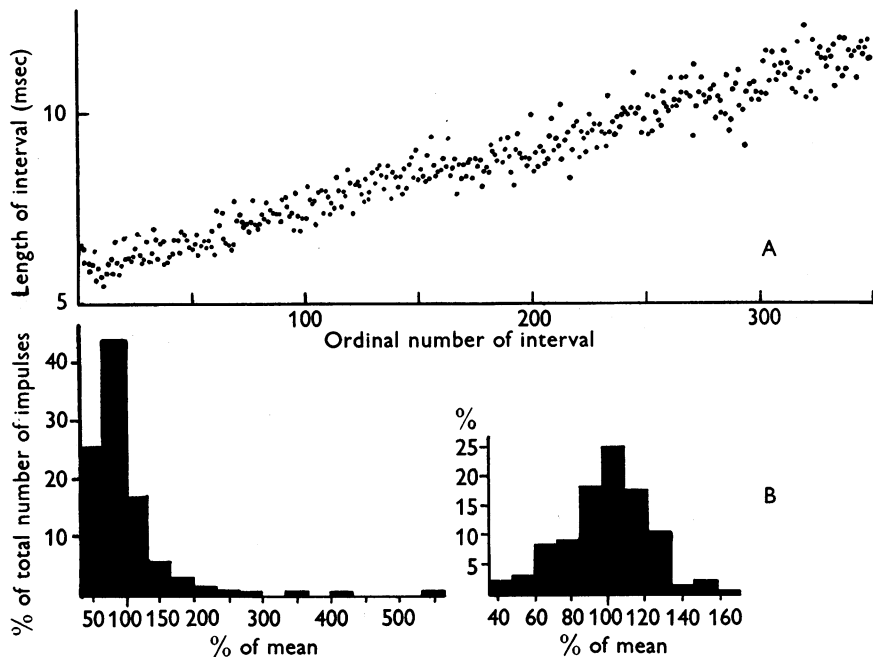


Fig. 1. A, adaptation of sensory discharge in a single axon, with the muscle held at constant length after rapid stretching. The change in interval (msec) between 350 successive impulses is shown. Abscissae: number of impulse. Ordinates: interval in msec. B, distribution curve for 130 intervals, mean value 107 msec (right), and for the reciprocal values of the same intervals (rate of discharge), mean value 11 imp/sec (left). Abscissae: percentage of mean value of interval and rate respectively. Ordinates: percentage of total number of intervals and impulses respectively.

have been allowed for by the laborious method of fitting a straight line or a curve to the changing intervals and calculating the s.d. about the curve. The latter procedure was adopted in a few cases to estimate the error involved in the alternative procedure of pooling twenty consecutive intervals. The error was found to be insignificant for mean intervals down to about 10 msec but was appreciable for shorter mean intervals, when adaptation became more marked. The tendency of the error was to give too large a s.d. by the method of pooling. Recordings showing very rapid adaptation at rates of 200–300 imp/sec were therefore not used in the statistical treatment.

Tests were carried out to see whether the irregularities of successive intervals were independent of the previous interval. Comparing the s.d. with a figure obtained from the difference of successive intervals from the preceding interval, the relationship remained constant.

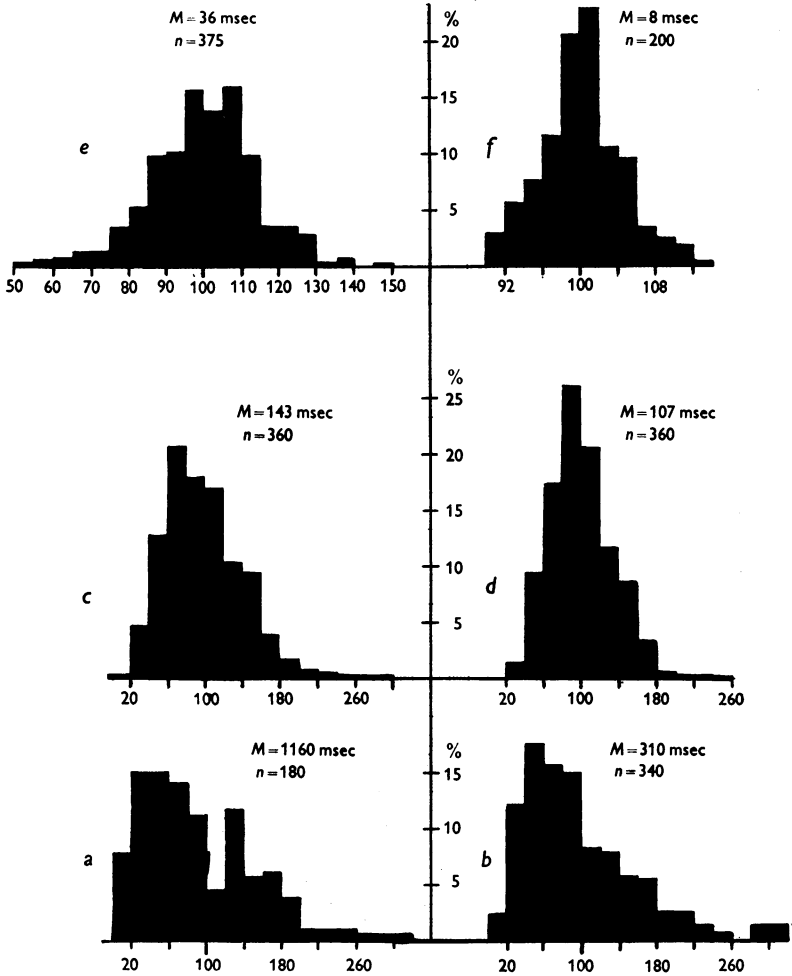


Fig. 2. Distribution curves for six groups of intervals, with mean values of (a) 1160 msec (180 intervals), (b) 310 msec (340), (c) 143 msec (360), (d) 107 msec (360), (e) 36 msec (375), (f) 8 msec (200). Abscissae: percentage of mean interval. Ordinates: percentage of total number of intervals. M = mean interval; n = no. of intervals.

The errors involved in measuring intervals and in assuming the paper speed to be constant during a recording were determined. The s.d. of intervals were corrected for these errors. The correction was quantitatively insignificant at mean intervals of 10 msec or more but then increased and became consider-

able at intervals less than 5 msec. In fact, these errors set a practical limit for the range of firing rates to be examined.

Experimental results. Our results are presented in Fig. 3, where the s.d. is plotted against the mean interval on a double logarithmic scale, and in Table 1. The results of the separate experiments were all plotted on the same graph without any individual scaling, the original values being used. A division into two groups of intervals was made, as shown in Table 1—those with intervals smaller than 160 msec and approximately symmetrical distribution curves

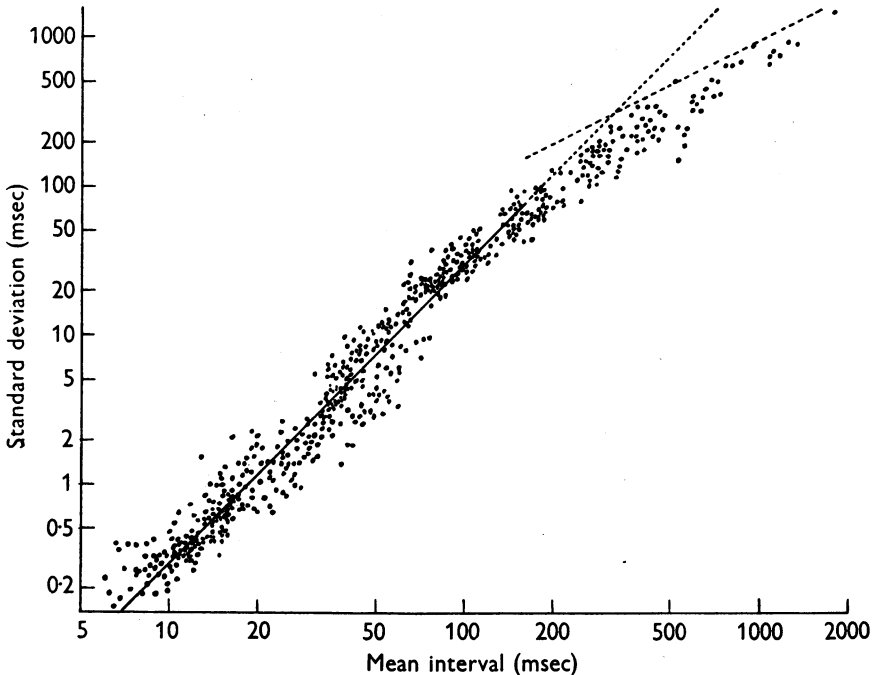


Fig. 3. Relation between mean interval in msec (abscissae) and s.d. in msec (ordinates) plotted on a double logarithmic scale. Each of the 590 points represents a pool of 20 intervals. A regression line, having a slope of 2.03, has been fitted to points with mean intervals less than 160 msec (for explanation see text), and the line, of slope 1.0, where the s.d. is equal to the mean, has been drawn for intervals greater than 160 msec.

(469 values), and those with intervals greater than 160 msec and asymmetrical distribution curves (121 values) (see Fig. 2). This division according to the shape of the distribution of intervals about their mean will be justified on theoretical grounds in the discussion.

In Fig. 3, to the group of intervals smaller than 160 msec, a regression line was fitted about which there is relatively little scatter. The slope of this line is 2 (2.03) which implies that the irregularity (s.d., msec) is proportional to the square of the mean interval (msec); also, as a natural consequence, the

irregularity expressed as imp/sec is approximately constant over this range, having a mean value of 3.50 ± 0.25 imp/sec for mean frequencies ranging from 7 to 163 imp/sec.

For asymmetrically distributed intervals the square relationship no longer holds, and with increasing intervals there is a tendency for the s.d. to approach and equal the mean, the slope in Fig. 3 approximating to unity; expressed as imp/sec the irregularity is no longer constant.

TABLE 1. Relation between mean and s.d. for intervals and frequencies. The table is divided into two parts, the upper representing 2420 intervals having asymmetrical distribution curves, the lower representing 9380 intervals having approximately symmetrical distribution curves.

Mean interval (msec) was converted to mean frequency (imp/sec) by taking the reciprocal. Half the difference between the reciprocals of (mean interval plus s.d.) and (mean interval minus s.d.) was taken as a measure of irregularity in terms of frequency. For simplicity, this quantity has been labelled 's.d. of frequency' in the table, although this is not a strictly correct term.

The over-all mean value of 's.d. of frequency' is 3.00 ± 0.23 imp/sec. The mean value for the lower group is 3.50 ± 0.25 imp/sec.

No. of pools of 20 intervals	Mean interval (msec)	Mean s.d. (msec)	Mean freq. (imp/sec)	Mean s.d. (imp/sec)
1	1800	1510	0.6	1.6
1	1320	906	0.8	1.0
5	1120	800	0.9	1.3
3	859	759	1.2	4.7
8	687	470	1.5	2.9
11	564	298	1.8	1.3
13	428	269	2.3	2.4
15	356	203	2.8	2.4
26	279	162	3.6	3.1
12	227	111	4.4	2.8
26	175	75	5.7	3.0
31	143	61	7.0	3.6
28	107	37	9.4	3.7
35	87	28	12	3.7
33	70	19	14	4.3
36	56	8.4	18	2.8
42	44	5.5	23	2.9
56	36	4.0	28	3.1
26	28	1.8	35	2.3
23	23	1.2	44	2.4
32	18	1.0	58	3.2
51	14	0.62	70	3.1
43	11	0.38	89	3.1
20	9.0	0.28	111	3.5
11	7.2	0.28	139	5.5
2	6.2	0.21	163	5.6

DISCUSSION

In order to evaluate the results the assumption is made that the rate of firing in a sensory axon from a frog's muscle is directly proportional to the degree of depolarization of its endings, between the limits of threshold and maximal depolarization. This assumption is the simplest possible one and is suggested

by the experimental observations of Katz (1950*b*, fig. 10). It can be expressed as

$$F = 1/T = k(E - E_0) \quad \text{for } E_{\text{max.}} > E > E_0, \quad (1)$$

with the differential

$$dT/dE = -kT^2, \quad (2)$$

where F = impulse frequency (imp/sec), T = time interval between impulses (sec), k = constant, E = depolarization (mV), $E_{\text{max.}}$ = maximal depolarization possible, and E_0 = threshold depolarization.

The hypothesis is that superimposed upon the steady level of depolarization there might be small fluctuations of fixed r.m.s. voltage due to thermal agitation of ions, or other causes in the nerve endings, these fluctuations being responsible for the irregularity in impulse initiation. In such a system when the axon is firing at very low frequencies with great irregularity, the depolarization level would be subthreshold and the initiation of impulses would be determined solely by random fluctuations momentarily rising to a value greater than threshold. Thus if depolarization were progressively reduced from just above threshold to below threshold, one would expect the initiation of an impulse to become more and more a matter of chance until finally it became purely random. The characteristic distribution curve for such a random sequence of intervals is an exponential one, for which the s.d. is equal to the mean (Feller, 1950; Fatt & Katz, 1952). In our experiments, for intervals longer than 160 msec, the distribution becomes more and more asymmetrical and the s.d. tends to equal the mean (Fig. 3). This fact provides the justification for dividing the observed values of intervals into two groups, the irregularity at low frequencies being explained as above.

It was observed that as long as the distribution of intervals is approximately symmetrical, the s.d. varies directly with the square of the mean interval, and expressed reciprocally, in impulses per second, the s.d. remains approximately constant (\pm imp/sec). The interpretation of this interesting result is not yet clear, but at first it appeared to support the idea that the observed fluctuations arise from a constant 'noise voltage' at the nerve ending. If there is a linear relation between the membrane potential and impulse frequency (eqn. 1) then a given small variation of potential ΔE would produce a constant variation of impulse frequency, independent of the mean discharge rate. (Expressed reciprocally in terms of intervals dT/dE would vary with the square of the interval (eqn. 2).)

Hence it appeared that one has to look for a voltage fluctuation of constant r.m.s. value, and this could well be thermal agitation noise in the nerve endings. Suppose the maximum depolarization which can be obtained is about 60 mV (cf. Hodgkin, 1951) and the corresponding maximum rate of discharge about 300 imp/sec (cf. Katz, 1950*b*) then, with direct proportionality, a variation of 3.5 imp/sec would correspond to a fluctuation of

membrane potential of about ± 0.7 mV. Fluctuations of this order of magnitude due to thermal noise might occur at exceedingly fine nerve terminals (below 0.1μ diameter) (cf. Fatt & Katz, 1952).

Unfortunately, the interpretation of our result is complicated by the following factors, which, at present, can only be stated in qualitative terms.

During stretch the resistance and capacity of the terminal nerve membrane are bound to change. The changes would have little effect on the r.m.s. value of the noise voltage. Supposing, for instance, the capacity increases by 20% during a 20% stretch, this would reduce the voltage noise by only 10% over the whole range of our observations. Similarly, a hundredfold reduction of membrane resistance such as might conceivably occur during depolarization (cf. Hodgkin, 1951) will effectively alter the r.m.s. value of noise not more than 10% provided that the membrane capacity remains constant (cf. Fatt & Katz, 1952). But while the r.m.s. value of the fluctuations would hardly change, its frequency spectrum would alter very considerably and spread to a greater extent over the higher range of frequencies during the stretched (i.e. depolarized) condition. This might give rise to a loss of physiologically effective noise because very brief fluctuations of membrane potential probably have little effect.

Secondly the effect which fluctuations of membrane potential have in accelerating or delaying the start of an impulse, cannot be assessed without knowing the 'frequency' as well as the r.m.s. amplitude of the fluctuations. As the frequency spectrum is likely to alter with stretch this must further affect the relation which we have studied.

Hence the interpretation of the result shown in Fig. 3 and the constant frequency fluctuation shown in Table 1, can hardly be as simple as at first appeared, though they might possibly arise from a cancellation of the complicating factors which we have just summarized.

During adaptation, the mean firing frequency decreases and the irregularity of discharge increases. It seemed possible, therefore, that adaptation might influence the irregularity in some specific manner. We could not find any such effect in several experiments where the frequency ranges of different recordings overlapped. The degree of adaptation, therefore, did not seem to affect the amount of irregularity for a given mean frequency, within the time period of adaptation studied in the present experiments.

The interpretation of the results is complicated by the presence of two variables whose significance cannot be assessed. Nevertheless, the result is clearly compatible with the possibility that the irregular disturbances in the initiation of afferent impulses have a constant source. One such source has earlier been suggested, viz. thermal agitation of ions at the finest nerve endings (Fatt & Katz, 1950, 1952).

SUMMARY

1. The irregularity in the discharge of impulses from a slowly adapting sense organ was investigated, using the muscle spindle of the frog.

2. A statistical analysis was made of the intervals between successive impulses over a wide range of mean intervals, varying between 1800 and 6.2 msec, at room temperature.

3. At very low discharge rates, successive intervals show an approximately exponential distribution, with the coefficient of variation tending towards a limiting value of unity, which is characteristic of a random sequence.

4. The fluctuation of the impulse frequency is found to be constant over a wide range, amounting to a standard deviation of 3.5 ± 0.25 imp/sec, for mean frequencies varying between 7 and 163 impulses per second. Furthermore, the s.d. of the impulse interval is found to be proportional to the square of the mean over a range of mean intervals between 143 and 6.2 msec.

5. The possible origin of the observed impulse fluctuation is discussed.

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