

## SPONTANEOUS SUBTHRESHOLD ACTIVITY AT MOTOR NERVE ENDINGS

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The present study arose from the chance observation that end-plates of resting muscle fibres are the seat of spontaneous electric discharges which have the character of miniature end-plate potentials. The occurrence of spontaneous subthreshold activity at an apparently normal synapse is of some general interest, and a full description will be given here of observations which have been briefly reported elsewhere (Fatt & Katz, 1950*a*).

### METHOD

The intracellular recording technique (Ling & Gerard, 1949; Nastuk & Hodgkin, 1950) was used in most experiments, confirmatory evidence being obtained with external recording in a few cases. The adaptation of the microelectrode technique to a study of the motor end-plate has been described in detail in a previous paper (Fatt & Katz, 1951).

The preparation was the *m. ext. longus dig. IV* and *m. sartorius* of the frog. In a few experiments, limb and abdominal muscles of lizards (*Lacerta dugesii*) and tortoises (*Testudo graeca*) were used.

### RESULTS

#### *Preliminary observations*

In the course of some earlier work, while recording from the surface of isolated muscle fibres, we occasionally noticed a spontaneous discharge of small monophasic action potentials. The potentials varied somewhat in size, but had a very consistent time course, rising rapidly in 1-2 msec, and declining more slowly, to one-half in about 3-4 msec. They were localized at one region of the fibre, and in their shape and spatial spread resembled the end-plate potential (e.p.p.) (cf. Eccles, Katz & Kuffler, 1941; Fatt & Katz, 1951). Moreover, the discharge disappeared when a moderate dose of curarine was applied to the muscle.

Not much attention was paid to the phenomenon at the time, and it was suspected to be due to a local injury discharge at the nerve endings, the axon having been cut close to the muscle fibre. In more recent experiments, how-

ever, we observed the same phenomenon in nerve-muscle preparations which seemed to be above any such suspicion, and this induced us to examine the nature of the discharge in detail.

*Localization of the spontaneous discharge at the end-plate region*

The formal resemblance of the spontaneous discharges to the e.p.p. suggests that they originate at the nerve-muscle junction. This is not by itself critical evidence, for any brief disturbance of the muscle membrane—anywhere along the fibre—would be followed by a local potential change similar in time course and spatial spread to the e.p.p. (see Eccles *et al.* 1941; Katz, 1948; Fatt & Katz, 1951). A more decisive point was that the discharge could never be seen at

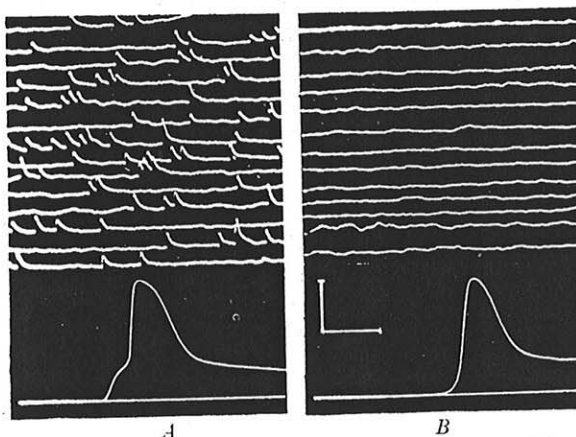


Fig. 1. Localization of spontaneous activity. M. ext. 1. dig. IV. Intracellular recording. Part A was recorded at the end-plate, part B at a distance 2 mm away, in the same muscle fibre. The lower part, taken at high speed and low amplification, shows the response to a nerve stimulus (shock applied at beginning of sweep); the upper part, taken at low speed and high amplification, shows the spontaneous activity at the end-plate. Voltage and time scales: 50 mV and 2 msec for the lower part, and 3.6 mV and 47 msec for the upper part.

nerve-free regions of the muscle fibre, and whenever the test was applied, its place was found to coincide exactly with that of the e.p.p. An example of this is shown in Fig. 1, where the response to a nerve impulse is recorded, together with the subthreshold activity, at two different points of a muscle fibre. The spontaneous discharge was seen at the 'end-plate', where the e.p.p. is large and the muscle spike originates, but not at a distance of 2 mm where the e.p.p. is small and the spike appears after a delay due to muscle conduction. In other experiments a large number of end-plates were mapped out in a curarized muscle, in the manner previously described (Fatt & Katz, 1951), and after removal of curarine the end-plates as well as nerve-free portions of the fibres were explored, by observing the membrane potential for periods of about 10-15 sec with high amplification. Experiments of this kind indicated that

the majority of the end-plates in normal, resting, muscle fibres of the frog are the seat of spontaneous miniature e.p.p.'s.

The term 'normal fibres' should perhaps be qualified, as we have studied the phenomenon only in isolated tissue. But it was seen in every (except denervated) muscle, and in fibres whose resting, action and end-plate potentials would be considered as 'normal'.

It might be argued that, by inserting a microelectrode at the end-plate, the nerve endings may be damaged or stimulated mechanically, and that this would lead to the observed local activity. This argument, however, is answered by the following finding: in many muscle fibres the occurrence of a spontaneous discharge was first detected at a distance of 1-1.5 mm from the end-plate, and only later was the electrode placed at the focal point where the potential changes were largest. Clearly, the miniature e.p.p.'s were present before the microelectrode approached the nerve endings sufficiently closely to be able to injure them. Damage to the nerve endings has apparently some potentiating effect (see p. 122 below), but it cannot be regarded as the cause of the phenomenon.

#### *Size and frequency of miniature end-plate potentials*

The amplitude of the miniature discharges varied from one fibre to the next, and even at a single end-plate the sizes of individual discharges were scattered over a fairly wide range around the mean (Fig. 13). Their order of magnitude was 0.5 mV (or approximately 1/100 of the size of the normal e.p.p., Fatt & Katz, 1950*a*, 1951). There were some differences between the two muscles

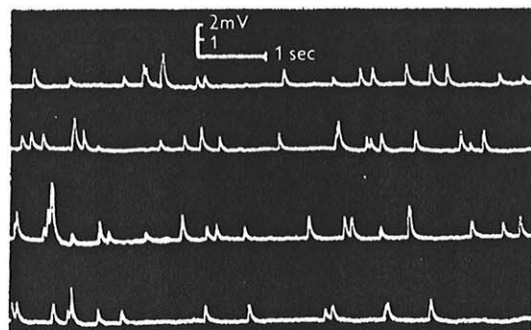


Fig. 2. Example of miniature e.p.p.'s in a muscle treated with  $10^{-6}$  prostigmine bromide.

which we studied most extensively: the potential changes were usually larger in the m. ext. l. dig. IV than in the sartorius, where they sometimes barely exceeded 0.1 mV. The largest individual amplitudes were obtained in prostigmine-treated muscle (Fig. 2, up to 3 mV), and much higher levels occurred during temporal summation of several discharges (cf. Fig. 8).

During any one experiment on a given end-plate, the *mean amplitude* remained fairly constant. The *mean frequency*, however, was unstable and subject to progressive change. In different fibres, often from the same muscles, frequencies varied over a thousand-fold range (between about  $\frac{1}{10}$  per sec and 100 per sec, at a temperature of about 20° C). The intervals between successive discharges, at any one end-plate, varied in a random manner which will be analysed in some detail below.

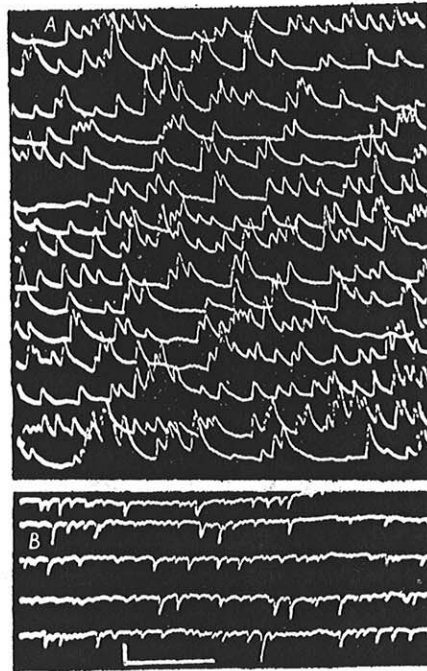


Fig. 3. 'High-frequency' discharge, recorded from the same end-plate as in Fig. 1, during a later stage of the experiment. *A*: microelectrode inside the fibre; *B*: microelectrode on the surface contacting an active patch (see text). Note: the polarity of the deflexion reverses when the electrode is on the outside. Scales: 1 mV and 50 msec.

#### *Active spots in the end-plate surface*

The results hitherto described were obtained with an intracellular electrode, with which the p.d. across the muscle fibre membrane was recorded. On a few occasions it was possible to locate one or two discrete spots on the fibre surface from which miniature e.p.p.'s of as much as 1-2 mV amplitude could be recorded externally, i.e. *without* penetrating the fibre and recording the resting potential. The discharges so recorded (e.g. Figs. 3-5) differed in certain respects from the 'internal' potential changes: (i) Their polarity was reversed, the microelectrode becoming negative with respect to the bath (with the electrode

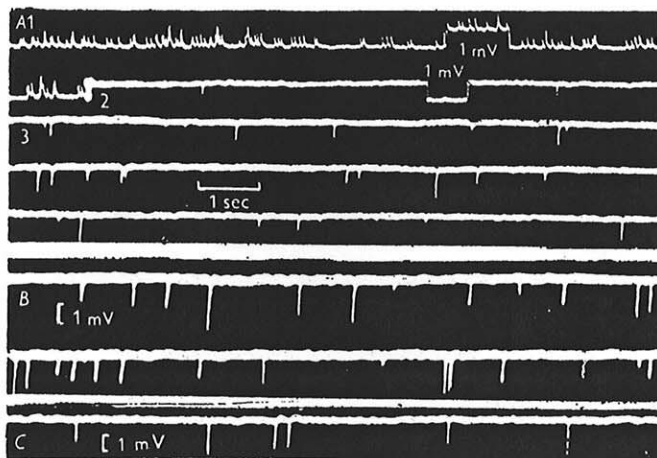


Fig. 4. 'Internal' and 'external' records. *A-C*: examples from three different end-plates (all from prostigmine-treated muscle). *A 1*: internal recording, with a 1 mV calibration step. *A 2* and *3*: external recording at two different spots on the same end-plate. *B* and *C*: external records from other end-plates.

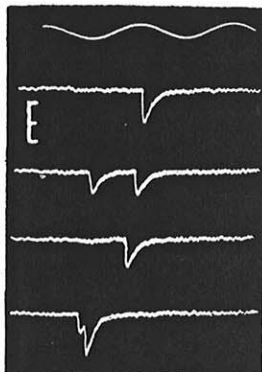


Fig. 5. External records from a single spot, using fast time base.  
Voltage scale: millivolts. Time: 50 c/s.

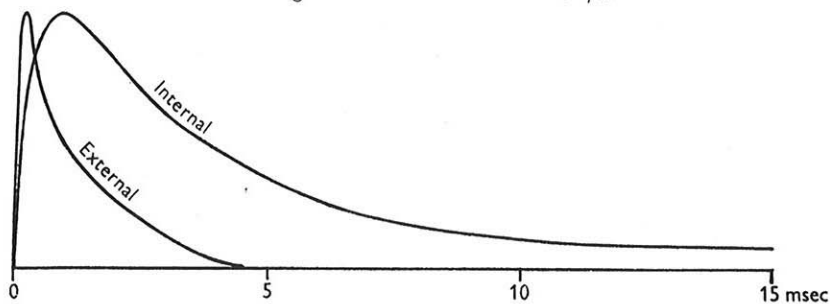


Fig. 6. Time course of internal and external miniature potentials taken from the experiment of Figs. 1 and 3. The two curves have been drawn to the same maximum, disregarding the opposite polarity of the records.

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*inside* the fibre, a steady resting potential of about 90 mV is obtained which diminishes during a discharge, i.e. the internal microelectrode becomes *less* negative). (ii) The localization of the external miniature potentials was extremely critical, the whole phenomenon vanishing when the microelectrode was moved some microns along the fibre surface. (iii) The external miniature e.p.p.'s had a very brief time course. For example, in the experiment of Figs. 3 and 6, the duration of rise and fall of the potentials was only about one-fifth of that internally recorded, and the decline terminated sharply giving the potential a characteristic 'triangular' shape. (iv) The size and frequency pattern of the external potentials was often quite distinct from those recorded internally, at the same end-plate. In Fig. 4, for instance, a few large discharges

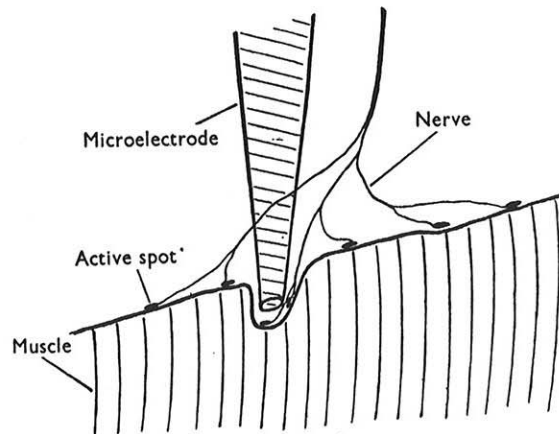


Fig. 7. Diagram to explain the characteristics of the externally recorded discharge.

are seen with the external electrode, their amplitude being more uniform and their recurrence rate much less than observed with the electrode inside the fibre. In other experiments, a few external potentials of large size were found to stand out among the remainder of the discharge which consisted of minute potentials more or less merging into the base-line noise.

It appeared in these cases that, with the microelectrode on the surface of the fibre, the recording of miniature e.p.p.'s became much more 'selective' and that all but a few active points of the end-plate, in closest proximity to the electrode, were 'rejected'. The diagram in Fig. 7 indicates a simple way of explaining the features of the external records. There are presumably a large number of active spots distributed within the end-plate surface at which individual units of the spontaneous discharge originate. With the electrode inside the fibre, the discharges of all these units are recorded without much discrimination, the separation of the active points being much less than the length constant of the fibre. But when the microelectrode is in the outside bath, no electric activity is seen unless the electrode tip happens to be placed

directly over one, or a few, of these active patches, in which case a relatively large external p.d. may be recorded from these units alone. This p.d. arises from the flow of current across the end-plate membrane and in the external fluid immediately surrounding the electrode tip, and the time course of these local currents (and therefore of the externally recorded potential) is considerably more rapid than that of the underlying membrane potential change (cf. Lorente de Nó, 1947; Fatt & Katz, 1951).

Finally, it might be questioned whether we are justified in attributing the external records to potential changes in the end-plate membrane (i.e. in the muscle), rather than to 'presynaptic' changes in the nerve endings. The fact that a moderate dose of curarine abolishes 'external' as well as 'internal' miniature potentials, strongly indicates that they both arise 'post-synaptically', in the end-plate.

#### *Effect of curarine and prostigmine*

The effect of these drugs on the e.p.p. is well known (Eccles *et al.* 1941) and forms an important part of the argument that the e.p.p. is due to a reaction between acetylcholine and the motor end-plate (Dale, Feldberg & Vogt, 1936). The spontaneous discharges are affected in the same manner: they are greatly reduced in size by curarine, and increased in amplitude and duration by prostigmine. A subparalytic dose of curarine (D-tubocurarine chloride  $5 \times 10^{-7}$ ) reduced the mean amplitude to about one-half, and after doses exceeding  $10^{-6}$ , the miniature discharges could no longer be seen. A concentration of  $4 \times 10^{-6}$  has previously been found to diminish the e.p.p. to between  $\frac{1}{4}$  and  $\frac{1}{8}$  (Fatt & Katz, 1951). A proportional effect would reduce the miniature potentials to little more than the noise level of the apparatus.

The effect of prostigmine is illustrated in Fig. 8. The increase in amplitude and duration resembles qualitatively that observed with the e.p.p. However, the effect shown in this figure is not as striking as the dramatic lengthening of the e.p.p. in the non-curarized state (Eccles, Katz & Kuffler, 1942; Fatt & Katz, 1951).

The prostigmine action had some interesting corollaries: (i) Successive miniature potentials summed to a higher level and occasionally gave rise to muscle impulses. (ii) The miniature e.p.p.'s, being larger and longer than in normal muscle, have a more effective electrotonic spread. This enables one to see the discharges at a distance of a few millimetres from the end-plate and makes their detection much easier.

It might appear from Fig. 8 that prostigmine increased the *frequency* of the discharge, but we were unable to find a statistically significant effect on frequency of either prostigmine or curarine ( $5 \times 10^{-7}$ ) in several other experiments.

*The effect of denervation*

The preceding experiments show that the spontaneous discharges are localized at the end-plates of resting muscle fibres. The time course of the individual potential indicates that it is due to a brief, impulsive, disturbance

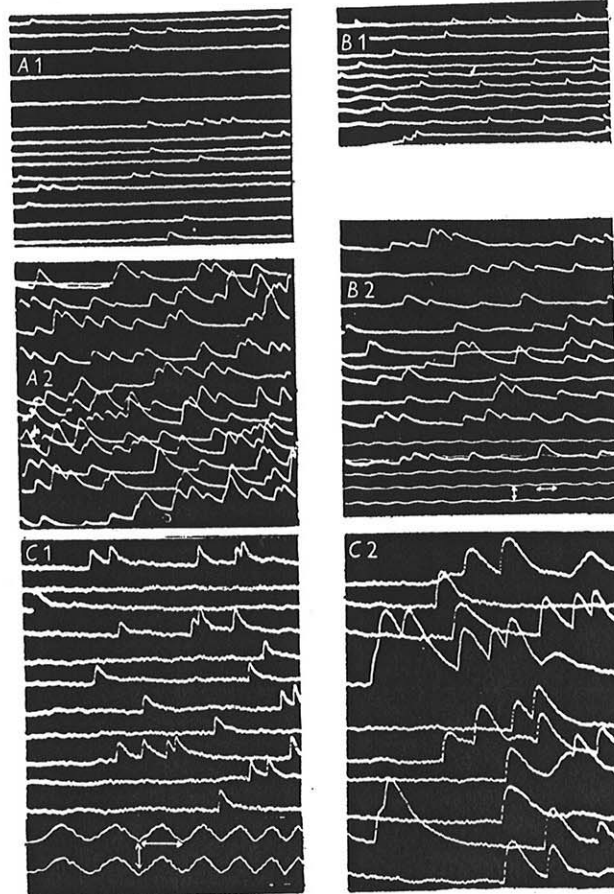


Fig. 8. Effect of prostigmine on the size and time course of the miniature e.p.p. *A-C*: three different end-plates (*A* and *C* from m. ext. 1. dig. IV, *B* from sartorius). 1: in normal Ringer; 2: after addition of  $10^{-6}$  prostigmine bromide. (In *C* 2, potential changes of slow rise can be seen, beside those of the usual rapid rate of rise. This was observed in several other experiments (cf. also Fig. 9*B*), and is presumably to be explained by electrotonic spread from an accessory end-plate on the same fibre.) Arrows indicate 1 mV scale and 20 msec (50 c/s).

of the membrane similar to the e.p.p., and the effects of curarine and prostigmine suggest that both arise from the action of ACh. A simple picture would be that at each nerve-muscle junction, the nerve terminals contain a large number of discrete 'active spots' at which ACh is released. The miniature potentials would be due to an asynchronous, spontaneous, activity of individual



spots, while the e.p.p. is due to a synchronous discharge of the whole apparatus. The e.p.p. could thus be regarded as the sum of miniature potentials produced by all the units.

While it seems very probable that the miniature e.p.p.'s are due to local discharges of acetylcholine, it may be questioned whether these discharges originate in the nerve endings. It would be conceivable, for instance, that stray amounts of ACh are released from vascular tissues surrounding the muscle fibres. Pertinent evidence on this matter was obtained by studying denervated muscles. The sciatic nerve of one leg was severed, and the frogs kept at room temperature. The two m. ext. long. dig. IV were isolated and tested more than 2 weeks later. Denervated frog muscle is known to become sensitized to ACh, while its nerve endings degenerate within a few weeks and lose their capacity of building up and releasing ACh (cf. Feldberg, 1943, 1945). The experimental result was clear: muscles whose nerves had been cut 2-3 weeks previously showed no miniature e.p.p.'s, while they were readily seen in the innervated companion muscles. The effect of denervation thus strongly supports the view that the discharges are due to a release of small quantities of ACh from functioning nerve terminals.

After more prolonged denervation, fibrillation gradually developed (cf. Reid, 1941). This was seen in only a few fibres of any one muscle, and in some experiments very infrequent subthreshold potential changes were observed in denervated muscle which may have been precursors of fully propagated fibrillation. These phenomena were too infrequent for any detailed study, but we noticed that the amplitude was highly variable (from less than 1 up to 10 mV) and the duration of the local potential change more prolonged than that of the miniature e.p.p.'s on the normal side. Moreover, the local changes as well as the fibrillation in denervated muscle seemed to be unaffected by curarine and prostigmine, and, in further contrast to the miniature e.p.p.'s (p. 120) were abolished by a small increase of calcium concentration (from 1.8 to 3.6 mM).

*Can the miniature discharges be attributed to molecular leakage of acetylcholine from nerve endings?*

ACh is synthesized continually by cholinergic nerves and nerve endings (Feldberg, 1945), and there is probably a slow continuous leakage of ACh molecules from this reservoir. The question arises whether such a leakage, i.e. a random collision of individual molecules of ACh with the end-plate, could produce the miniature e.p.p.'s. It would be difficult to explain the effect of prostigmine on such a 'single-molecule' hypothesis, but there are two more cogent arguments which indicate that the miniature potentials must be due to the synchronous release of a large number of acetylcholine molecules and cannot be explained by a mechanism of simple molecular diffusion.

(i) The quantity of ACh released by an impulse at one end-plate has been estimated (cf. Acheson, 1948; Rosenblueth, 1950) as being of the order of  $1.5 \times 10^{-16}$  g or about  $10^6$  molecules. This value was derived from perfusion experiments with prolonged stimuli, in which large losses must have occurred, and the true amount released locally during the first impulse was presumably

much larger. But even if we accept this estimate, the amount responsible for a miniature e.p.p. would be of the order of 1/1000–1/100 of that for an e.p.p., which is still an aggregate of thousands of ACh molecules.

(ii) If the miniature discharges were to be regarded as the local depolarizing effect of individual ACh molecules then, by the same argument, the application of ACh in solution should greatly increase the frequency of the miniature discharges; in fact, the steady depolarization which is produced by applied ACh would have to be regarded as a fusion of miniature e.p.p.'s, like the fusion of twitches in a tetanus.

This conclusion, however, is contrary to our observations. A moderate concentration of ACh ( $5 \times 10^{-8}$ , with  $10^{-6}$  prostigmine bromide) which depolarized the end-plates by a few mV, did not appreciably alter the frequency of the miniature discharges. The only noticeable change was a slight reduction of amplitudes, similar to the reduction of the e.p.p. observed at depolarized end-plates (Nastuk, 1950; Fatt & Katz, 1951). These results seem to us to be incompatible with the suggestion that the miniature potential might be attributed to the action of one (or very few) ACh molecules; if individual molecular collision between ACh and the end-plate builds up a steady depolarization then the molecular units of this depolarization must be much smaller than the recorded miniature e.p.p.

*Miniature end-plate potentials and spontaneous excitation at  
motor nerve terminals*

On the basis of the preceding argument it can be said that a miniature e.p.p. is due to a spontaneous momentary release of many molecules of ACh from a small area of the motor nerve endings. The quantity may be estimated as being rather less than 1/100 of that during the transmission of a motor nerve impulse. Successive miniature e.p.p.'s are of uniform shape, though of variable amplitude (Fig. 13) and follow one another in a random sequence (p. 122).

Suppose the motor axon has about 100 branches, or alternatively, that there are within the terminal area some 100 discrete 'patches' concerned with the release of ACh. If these terminal structures have a special tendency to spontaneous excitation, then our observations would be easily understood. It has been pointed out (Fatt & Katz, 1950*a*) that spontaneous excitation might simply be the result of excessive voltage noise across the nerve membrane, and that this noise level is likely to be largest at the smallest nerve endings. This idea, which was originally suggested to us by Mr A. L. Hodgkin, is discussed more fully below. Whether it provides the complete explanation or not, the present experiments make it very likely that spontaneous excitation at individual motor nerve endings does occur and that it is the immediate cause of the miniature e.p.p.'s. In addition to the above results, the following observations are of interest in this connexion.

(i) Agents which are known to abolish electric excitation also extinguish the spontaneous discharge. Thus, the miniature e.p.p.'s cannot be seen in sodium-free solutions (made by substitution of isotonic sucrose), or in Ringer solution to which a small dose of a local anaesthetic (0.01% novocaine) has been added. Partial withdrawal of sodium (see below), or low concentrations of novocaine (0.001–0.002%) cause the amplitude of the miniature potentials to diminish. These results are interesting, though not unequivocal, for withdrawal of sodium has, and application of novocaine may have, some curare-like action on the end-plate (Fatt, 1950), quite apart from affecting nerve excitability.

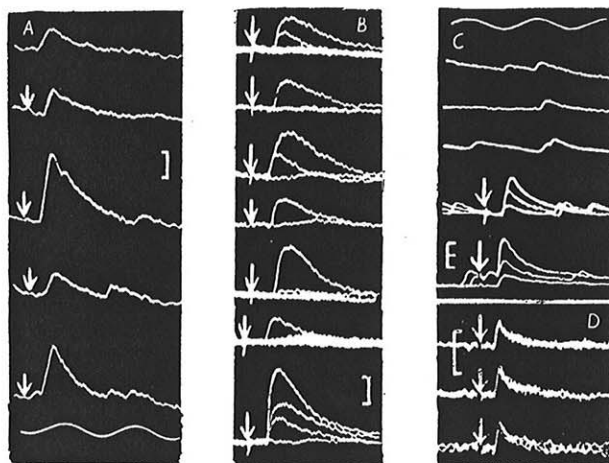


Fig. 9. *Effect of Ca-lack.* A–C: three different experiments. Muscles soaked in reduced ( $\frac{1}{4}$ ) Ca concentrations; in B, prostigmine bromide  $10^{-6}$  was present. Note: the records in A and the three top records in C were single sweep records. All other records were obtained with multiple sweeps repeated at about 1 per sec, during each of which the nerve was stimulated (at the instant marked by an arrow), though there was not always an end-plate response (B). The top record in A, and the three top records in C show spontaneous discharges only. All other records show e.p.p. responses to nerve stimulation, varying in step-like manner between zero and a few millivolts (e.g. B, bottom record). In some records (in A and C) spontaneous discharges are seen on the same sweep, immediately before or after the e.p.p. response. For comparison with the effect of Ca-lack, the relative constancy of the e.p.p. response in a curarized fibre is shown in D ( $5 \times 10^{-6}$  D-tubocurarine chloride; three successive records, each with three superimposed sweeps). Volt scale: millivolts. Time: 50 c/s.

(ii) A curious effect was observed when reducing the calcium concentration. This causes the size of the e.p.p. to diminish *without* affecting the size of the miniature potentials. With a sufficiently low calcium level, the response to a motor nerve impulse may be no larger than a single miniature e.p.p. In this condition, successive nerve impulses evoke a random display of minute e.p.p.'s whose sizes vary in a step-like fashion, seemingly corresponding to multiples

of a miniature discharge (cf. Fig. 9 *B*, bottom record, where steps of 0, 1, 2 and 3 can be recognized). In the experiment of Fig. 9 *B*, 328 strong shocks were applied to the nerve, 188 of which failed to elicit an end-plate response. Of the observed 140 e.p.p.'s, 100 had an amplitude which was within the range of the spontaneous miniature potentials (mean of 33 spontaneous discharges: 0.87 mV, standard deviation  $\pm 0.18$  mV), while the residual 40 e.p.p.'s were greater (up to 2.92 mV) and presumably represent responses of two or three miniature units. The contrast between this behaviour and the relatively constant response of a deeply curarized muscle fibre is illustrated in Fig. 9 (*A-C* with low calcium, *D* with curarine). The experiment throws some new light on the action of calcium at the nerve-muscle junction: lack of calcium apparently reduces the e.p.p. in definite 'quanta', as though it blocks individual nerve terminals, or 'active patches' within them, in an all-or-none manner. The normal e.p.p. can be seen, as in Fig. 9 *A-C*, to break down into individual miniature units. Conversely, it may be said that an individual spontaneous discharge does not differ, in appearance, from the response of a terminal unit to the motor nerve impulse.

#### *The effect of sodium and calcium ions*

Variations of Na or Ca concentrations have very similar effects on the e.p.p. (Fatt & Katz, 1950*b*; Castillo-Nicolau & Stark, 1951). With both ions the e.p.p. decreases as the concentration falls, the relation being approximately proportional over a certain range.

The effects of these cations on the miniature potentials, however, are different: the size of the miniature e.p.p. falls as the Na concentration is lowered, but it is independent of Ca concentration (see preceding section). For example, a reduction of Na concentration to one-quarter diminished the mean size of the miniature e.p.p.'s in one experiment from  $2.28 \pm 0.05$  to  $0.82 \pm 0.03$  mV, in another experiment from  $0.72 \pm 0.03$  to  $0.31 \pm 0.01$  mV (prostigmine being used throughout). The average reduction of the mean amplitude in several experiments with one-quarter Na was to 0.4, while the resting potential remained constant, at about 90 mV.

A reduction of Ca concentration to one-quarter had no significant effect, the mean size of the miniature potentials increasing, on the average to 1.01 (s.e. of mean of five experiments was  $\pm 0.02$ ). A fourfold increase of Ca concentration produced a small diminution of the miniature potential, on the average to 0.89 (s.e.  $\pm 0.02$ , five experiments).

There are reasons for supposing that both species of cations play a specific part in the release of ACh from active nerve endings, and the present observations are likely to be relevant in this connexion. A full discussion of this matter will be deferred until a later paper.

As regards the mean frequency of the spontaneous discharges, Na and Ca

have no clear-cut effect. In some experiments lack of Ca reduced the mean frequency appreciably, but the effect was not consistently observed, and in several later experiments, no change was seen. Lowering the Na concentration to one-quarter did not significantly alter the recurrence frequency.

*Agents which have a pronounced effect on the recurrence frequency*

The changes in the spontaneous discharge which have been described concerned the *size* of the miniature potentials, while little or no effect on the mean frequency was found. Because of its gradual uncontrolled variations, the frequency cannot be measured accurately over a long period, and it is only possible to look for gross changes.

Of the various agents studied, changes of temperature and of osmotic pressure were found to produce marked effects on the frequency of the discharge.

*Temperature.* In these experiments, the bath was replaced at intervals by Ringer's solution of different temperature (varying between 8 and 25° C), and records of spontaneous discharges were obtained from a number of initially located end-plates. The frequency increased with temperature, corresponding to a positive temperature coefficient with a  $Q_{10}$  of about 3. It should be noted that we have only studied the *steady* effect; our procedure did not allow us to observe initial transient changes which might conceivably have a different, or even a negative, temperature coefficient (see Sand, 1938).

*Osmotic pressure.* The most dramatic effect was observed when the osmotic pressure of the bathing fluid was changed (cf. Fig. 10). The addition of a small quantity of sucrose or NaCl to the bath causes a striking increase in the frequency of the discharge, and conversely dilution of the Ringer's fluid reduces the frequency. For example, a 50% increase of tonicity (by addition of sucrose) was followed by a reversible 45-fold increase of discharge frequency

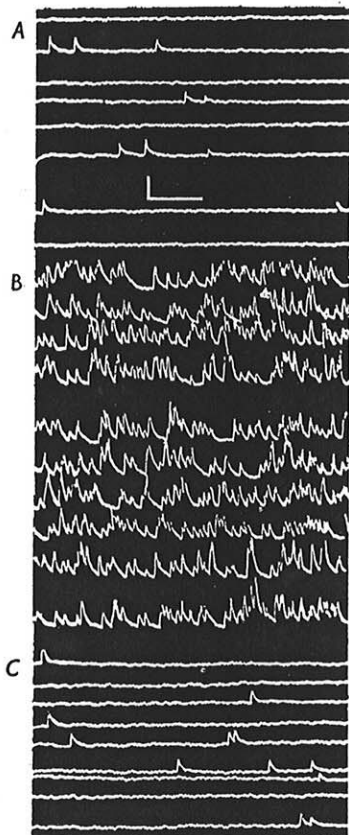


Fig. 10. Effect of osmotic pressure on discharge rate. *A* and *C*: normal Ringer, before and after applying (in *B*) a 50% hypertonic solution. Scales: 1 mV and 0.1 sec.

(from 2 to 90 per sec). In other experiments, raising the osmotic pressure by 30% caused the frequency to increase from 15 to 150 per sec, and lowering the osmotic pressure by 50% reduced the frequency from 28 to 0.9 per sec.

Finally, there was some indication that damage to the nerve endings is followed by an increase in the frequency of the discharge. In the course of repeated 'focal' insertions of the microelectrode, we observed sometimes that the e.p.p. response to a motor impulse progressively diminished (while the resting potential was well maintained), and at the same time the miniature potentials became progressively more frequent. Stretching a muscle 10-15% beyond its resting length reversibly increased the rate by a factor of 2.5-3, and pulling on the motor nerve sometimes caused a similar 'speeding up' of the discharge. It is possible that these procedures produce some local depolarization and thereby increase the instability of the nerve endings. There are however no means at present of obtaining direct evidence of the state of the nerve terminals and of their membrane potential.

*The 'random' nature of the spontaneous discharge*

The sequence of miniature potentials appeared to be completely irregular. It is true that, occasionally, this irregularity was broken by a short burst of high-frequency discharges, which might have been due to an extraneous stimulus or more probably to interaction between the different contributing units, e.g. to electrotonic currents from an active nerve ending lowering the threshold of adjoining endings. There were, however, long periods during which no bursts could be seen, and this suggests that any 'coupling' between the various units must be very weak and only rarely and temporarily leads to effective interaction.

The random succession of the discharge was subjected to a statistical test. It is a characteristic property of this type of random time series that the probability of occurrence of any one event does not depend upon past history. For an interval  $\Delta t$ , which is very brief compared with the mean interval  $T$ , the probability  $P$  of at least one occurrence is simply  $\Delta t/T$  (for a complete theory see Feller, 1950, chapter 17). As the interval  $t$  becomes greater,  $P$  increases exponentially according to the equation  $P = 1 - \exp(-t/T)$ . Similarly, the intervals between successive discharges, in a very large series of observations (total number  $N$ ), should be distributed exponentially, the frequency of occurrence  $n$  of any interval between  $t$  and  $t + \Delta t$  being given by  $n = N\Delta t/T \exp(-t/T)$ . To test the applicability of these equations, a series of 800 miniature potentials, covering a total period of 176.8 sec (i.e. with a mean frequency of  $800/176.8 = 4.52$  per sec) was chosen. This series was selected because it showed no obvious bursts of synchronized activity, and the mean frequency was suitable for accurate measurement (at too high frequencies, summation and coincidences between several unitary discharges make measurements uncertain; at too low

rates, the experiment becomes too long and progressive changes of the mean are likely to occur). To check that the mean frequency had not appreciably altered during the period of observation, the measurements were carried out separately for two series of 500 and 300 discharges respectively, and these agreed satisfactorily.

Successive intervals between individual discharges were divided into groups, as in Fig. 11. The distribution fits a simple exponential curve

$$n = N\Delta t/T \exp(-t/T),$$

where  $T$  is the mean interval ( $\chi^2 = 27.9$ ,  $f = 21$ ). If we plot the *total* number of intervals, whose duration is smaller than  $t$ , against  $t$ , as in Fig. 12, the results are seen to fall closely along the predicted curve  $P = 1 - \exp(-t/T)$ .

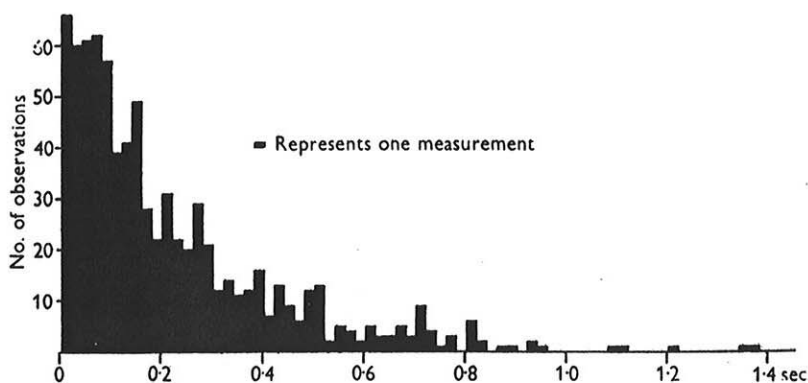


Fig. 11. Distribution of time intervals between discharges, in a series of 800 miniature potentials. The shortest interval which could be resolved in this experiment was 5 msec (the interval between 0 and 0.005 sec has been left blank). The observations were grouped in classes of 20 msec. The mean interval was 0.221 sec.

This random sequence of miniature potentials should, however, be interpreted with some caution. It evidently means that in this particular set of observations there was no noticeable interaction between the various contributing units, but it does not prove that the constituent units themselves discharged in a completely random manner. The record of impulses from a whole sense organ may appear to be chaotic, yet each nerve unit carries impulses of remarkable regularity. Whether such regular rhythms are concealed in the present picture of miniature e.p.p.'s cannot be said, because it has not yet been possible to isolate the constituent units satisfactorily. Selective recording from critical surface spots (cf. p. 112) might provide the answer. Our external records did not show any regular rhythmicity, but they were too few to provide a conclusive test.

*Statistical distribution of amplitudes*

We have suggested that there are a number of individual units (nerve terminals, or discrete 'ACh-release patches' within them) which independently contribute to the spontaneous discharges, and one might expect to find a grouping of the individual sizes corresponding to such units. In some experiments, this appeared to be the case, especially when external records were obtained (p. 114). However, when a large series of measurements was tested

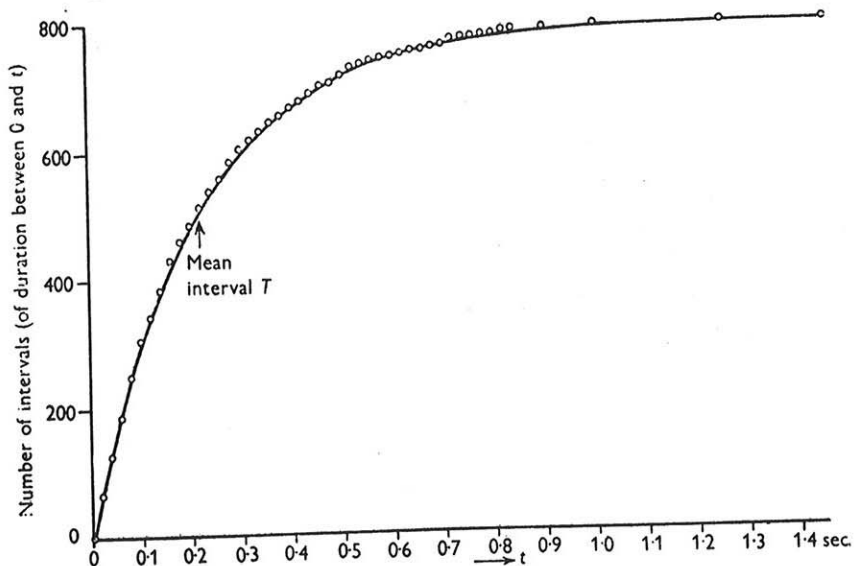


Fig. 12. Total number of observed intervals, of duration less than  $t$  (from the experiment of Fig. 11), has been plotted against interval duration  $t$ . Circles: observed numbers. Full line: a theoretical curve,  $y = N[1 - \exp(-t/T)]$ , where  $N$  is total number of observations, and  $T$  the mean interval, marked by arrow.

as in Fig. 13, the sizes were usually found to be scattered in an approximately normal manner around a simple mean value. In Fig. 13 the coefficient of variation was about 30%, and there were no outstanding secondary humps or peaks. The explanation of this scatter is probably several fold: (i) the mean amplitude of the individual potentials is about 1/100 of the e.p.p., hence some 100 different units may be involved, and a discrimination of individual sizes would then be impossible; (ii) the size of an individual unitary discharge must itself be subject to some variation, depending upon the interval between discharges (both 'facilitation' and 'depression' may be expected, as with two successive e.p.p.'s; cf. Eccles *et al.* 1941); (iii) the accuracy of individual measurements is limited by a random error of a few per cent, due to the baseline noise. These factors might easily account for the statistical spread of amplitudes shown in Fig. 13.



Perhaps the most convincing evidence for the existence of discrete terminal units is that obtained in calcium-deficient muscle (Fig. 9), where it was shown on p. 119 that successive e.p.p. responses varied in a step-like manner, corresponding to units of miniature e.p.p.'s.

In Fig. 13 there is indication of several discharges of about twice the mean amplitude, and of one isolated discharge of three or four times the mean size. These are very probably examples of a 'coincidence' of two (or three) unitary discharges which could not be resolved with the slow film speed used in this experiment. In fact, the smallest interval that could have been detected is

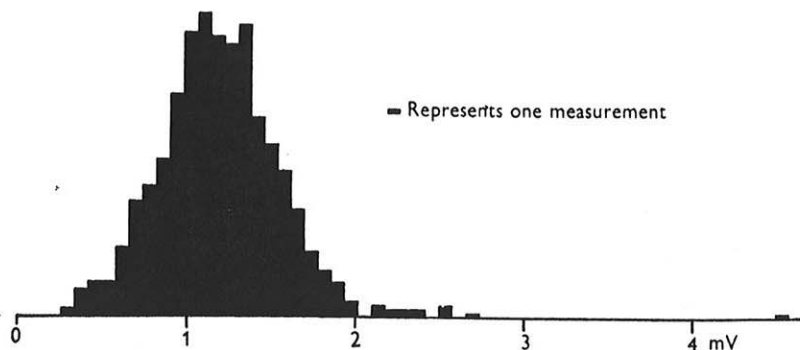


Fig. 13. Distribution of amplitudes. Muscle treated with prostigmine. Same 800 miniature potentials as used in Figs. 11 and 12.

about 5 msec. The chances of such small intervals occurring are given, approximately, by  $\Delta t/T$ , which is  $5/221 = 1/44$ . Hence, in 800 observations, eighteen 'coincidences' may on the average be expected. The chance that two such intervals follow one another is only  $1/44^2 = 1/1936$ . Thus, it is reasonable to suppose that the single observation of 4.6 mV amplitude was a 'triple' coincidence while the thirteen observations between 2 and 3 mV were ordinary 'double' coincidences.

#### DISCUSSION

The results point to the conclusion that some terminal spots of the motor nerve endings are spontaneously active and release ACh in the same impulsive manner as they do after the arrival of a normal motor nerve impulse.

The question arises why such 'neurogenic' activity does not lead to a back-firing into the main motor axon and, by axon reflex, to a total discharge of the motor unit. The answer is probably that the terminal area which, at any time, is spontaneously active is too small to produce a propagating nerve impulse. It should, however, be noted that in the eserinated mammalian muscle spontaneous 'fibrillation' (fibre activity) and 'fasciculation' (motor unit activity) have both been described (Masland & Wigton, 1940), which indicates that under certain conditions a back-firing into the motor nerve axon does, in fact, occur. While in most of our experiments the level of the spontaneous activity

remained below the threshold of either nerve or muscle fibres, in some prostigmine-treated muscles occasional fibrillation was seen which was presumably due to a summation of miniature potentials exceeding the threshold of a muscle fibre. It is likely that the spontaneous twitching in eserized or tetanus-poisoned mammalian muscle arises from the same phenomenon.

That the occurrence of miniature e.p.p.'s is not an exclusive property of frog's muscle was made evident by a few tests on lizard and tortoise preparations in which the same type of spontaneous activity was found in innervated regions of muscle fibres.

The second problem which confronts us is the origin of the spontaneous excitation at nerve terminals. In a previous note (Fatt & Katz, 1950*a*) it has been suggested that the 'noise' voltage across the axon membrane may become so large, at a sufficiently minute structure, that it may occasionally exceed the threshold level at some point. By 'noise' voltage is meant the random fluctuation of the resting potential due to thermal agitation of ions within the membrane.

This noise level may be calculated from the formula

$$E^2 = 4kT \int_0^{f_1} r df \quad (1)$$

(see Campbell & Francis, 1946), where

$E$  = r.m.s. value of noise fluctuation (volts);

$k$  = Boltzmann's constant =  $1.38 \times 10^{-23}$  joules per degree Kelvin;

$T$  = absolute temperature (degrees Kelvin);

$r$  = resistive component of impedance across nerve ending (ohms);

$f_1$  = frequency range over which the effective noise energy is distributed (c/s).

The resistive component  $r$  at the end of a long non-medullated axon is twice as large as in its middle, and is given by

$$r = a\sqrt{r_i r_m},$$

where

$$a = \sqrt{\left\{ \frac{1}{2} \left[ \frac{1}{1 + \omega^2 \tau_m^2} + \frac{1}{\sqrt{1 + \omega^2 \tau_m^2}} \right] \right\}},$$

$$\omega = 2\pi f,$$

$$\tau_m = r_m c_m.$$

and  $r_m$ ,  $r_i$  and  $c_m$  are, respectively, the transverse membrane resistance, the longitudinal axon cylinder resistance and the membrane capacity of one centimetre length of fibre (or terminal branch). These values are related to the more fundamental fibre constants as follows:

$$r_m = R_m / \pi d, \quad r_i = 4R_i / \pi d^2, \quad r_m c_m = R_m C_m,$$

where  $d$  is fibre diameter (cm),  $R_m$  specific membrane resistance ( $\Omega \times \text{cm}^2$ ),  $R_i$  specific axoplasm resistance ( $\Omega \times \text{cm}$ ), and  $C_m$  specific membrane capacity ( $\mu\text{F}/\text{cm}^2$ ). Equation (1) can, therefore, be rewritten as

$$E^2 = \frac{8kT}{\pi} (R_i R_m)^{0.5} d^{-1.5} \int_0^{f_1} a df,$$

from which it is seen that the noise voltage increases as the fibre size diminishes.

The absolute values can only be guessed, as we have no direct information about the size and electrical characteristics of nerve endings. Supposing that the fundamental electrical constants of nerve endings are of the same order of magnitude as in several types of non-medullated axons, we may use the following values:  $R_m = 5000 \Omega \text{ cm}^2$ ;  $R_i = 200 \Omega \text{ cm}$ ;  $C_m = 1 \mu\text{F}/\text{cm}^2$ . In computing  $\int a df$ , an arbitrary upper limit of integration ( $f_1$ ) had to be chosen; we assumed that the upper

frequency limit of effective noise components is at about 10 kc/s, and that at frequencies above it the fluctuations of membrane potential would be too rapid to lead to local excitation. On these assumptions,  $\int a df$  is calculated to be approximately 800 c/s.

The noise level, for an axon of these properties, depends then only on its diameter, the noise voltage increasing as the fibre size is reduced. For a 100  $\mu$  axon, for instance,  $E$  is about 2  $\mu$ V; for the terminal of an extremely fine fibre (or sufficiently long branch) of, say, 0.1  $\mu$  diameter,  $E$  becomes about 0.5 mV. These are r.m.s. values; the peak amplitudes of the fluctuation are several times larger, but they become very infrequent above 3-4 times the r.m.s. value, and in practice will not exceed five times this size.

The result of this calculation remains indecisive: it indicates that at very fine nerve terminals, thermal agitation noise may reach an amplitude of 1 or 2 mV and thereby a physiologically important range; nevertheless, on the present assumptions, there is little chance of the membrane noise approaching the 15-20 mV which is the threshold level of a *Loligo* axon (Hodgkin, Huxley & Katz, 1949). Although thermal agitation of ions may play an important part in the production of the discharge, some other property of the nerve endings, as yet unspecified, may well be involved.

#### SUMMARY

1. End-plates of many resting muscle fibres are the seat of spontaneous subthreshold electrical activity. It consists of a random succession of miniature end-plate potentials, their amplitude being of the order of 1/100 of the normal end-plate response to a motor nerve impulse.

2. The miniature potentials are greatly reduced in size by a small dose of curarine, and are increased in size and duration by prostigmine. They are abolished by denervation, and by nerve anaesthetics.

3. The frequency of the discharges varies over a wide range. It increases with temperature and, strikingly, with small increases of osmotic pressure.

4. There is evidence that the discharges are due to spontaneous local excitation of individual motor nerve endings, or of even smaller specialized membrane areas which are concerned with the release of acetylcholine.

5. The effect of sodium and calcium ions has been studied. Calcium deficiency is known to reduce the size of the end-plate potential (e.p.p.); this effect appears to take place in 'steps', involving an all-or-none blockage of a variable number of miniature components. The size of individual miniature potentials in contrast to that of the e.p.p. is not affected by calcium-lack. Sodium deficiency, on the other hand, reduces the amplitudes of both e.p.p. and miniature potentials.

6. The origin of the spontaneous discharge is discussed. It is possible that thermal agitation of ions across the nerve membrane plays an important part in their initiation.

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