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THE QUANTAL NATURE OF TRANSMISSION AND SPONTANEOUS MINIATURE POTENTIALS AT THE CRAYFISH NEUROMUSCULAR JUNCTION

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Of all the synapses that have been studied the physiology of neuromuscular transmission in vertebrates is most advanced, mainly as a result of the more recent microphysiological work by del Castillo, Fatt and Katz (Katz, 1958). The chemical nature of the transmission process having been established earlier, they found that transmission occurs by the simultaneous release of many packets or quanta of acetylcholine (ACh) from the motor nerve terminals. These quanta, each of which consists of many molecules of transmitter, cause in the post-synaptic membrane the end-plate potential. This sets up conducted impulses in twitch muscle fibres. Single quanta are also released spontaneously in a random sequence in 'resting' muscle.

In the crayfish and other crustacea the excitation process differs from that in vertebrates in many respects. The motor innervation is widely distributed over the muscle-fibre surface (van Harreveld, 1939; Wiersma, 1941). In many muscles the graded junctional potentials only rarely cause conducted impulses (Wiersma, 1941; Katz & Kuffler, 1946). Accordingly, much of the normal contractile activity in individual muscle fibres is graded. Further, the transmitter is different from that in vertebrates and most crustacean muscles also have an inhibitory innervation. In the face of these differences it was of interest to see if any evidence could be found that the mechanism of transmitter release in crayfish was similar, i.e. whether it also occurred in quantal steps.

This is the first of a series of papers in which we report on the quantal nature of transmission in the crayfish, how facilitation is linked with this quantal release of transmitter and how inhibition interacts with it. A preliminary note on spontaneous miniature potentials has already appeared (Dudel & Orkand, 1960).

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METHODS

Almost all experiments in the current studies were done on the abductor muscle of the dactyl (corresponding to the opener of the claw) in the 1st or 2nd walking leg of the crayfish *Orconectes virilis* (Steinhilberi). The muscle is relatively thin, consisting of several layers of short fibres which originate on the exoskeleton and are inserted on a central tendon. Most of the fibres which were used were 200–300 μ in diameter and about 2–3 mm long in the relatively small animals. The adductor of the dactyl was removed, leaving the flat inner surface of the abductor exposed. The intact exoskeleton around the muscle formed a natural chamber with a volume of not more than 0·1 ml. The leg was placed in a Lucite chamber, with the dactyl and the carpopodite fixed (Fig. 1). The inhibitory and excitatory axons to this muscle are contained in separate bundles which were exposed in the meropodite. The bundles were kept submerged in the second compartment of the chamber and were stimulated with fluid electrodes. These consist of glass tubes with a small opening, large enough for a nerve bundle to go through, connected to a syringe by fine tubing. Various lengths of nerve can be pulled up into the tube, together with physiological solution. The stimulus is then applied between the inside and outside of the glass tube.

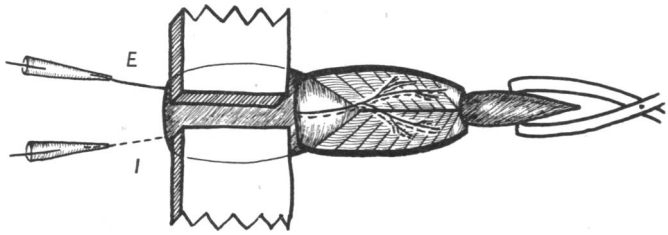

Fig. 1. Scheme of the preparation of the abductor of the dactyl in the crayfish, viewed from above. Adductor muscle removed. E = excitatory axon, I = inhibitory axon.

The composition of van Harreveld's solution (1936) is (mM) NaCl 195, KCl 5·4, $CaCl_2$ 13·5 and $MgCl_2$ 2·6. The pH was kept near 7·5 with 10 mM tris maleate buffer. Most experiments were done at 21–23° C. The muscle fibres were kept covered by physiological solution and the fluid was periodically replaced by adding a few drops of fresh solution. Alternatively a constant stream of fluid was kept flowing past the muscle fibres.

The recording system consisted of conventional DC and AC amplifiers. The micropipettes were generally filled with 3M-KCl and had resistances of 7–20 MΩ. Their tip diameters were 1 μ or smaller. For exploration of the potentials along the fibre surface, larger pipettes (1–5 MΩ) were sometimes used, filled with saturated NaCl solution and having tips of up to several micra diameter.

Muscle fibres were viewed under dark-field illumination, which enabled one to see the course of nerve branches. The finer ramifications near the junctions were not visible. The excitatory and inhibitory axons run together and also branch in unison innervating fibres at multiple spots (van Harreveld, 1939; Fatt & Katz, 1953).

RESULTS

Spontaneous miniature potentials

If one inserts a micro-electrode into the abductor muscle fibres anywhere along their course, one generally observes, in the absence of nerve stimulation, small potential changes of variable size, as shown in Fig. 2*A*. They

usually occur at frequencies around 1/sec but in some preparations they are seen only once every few seconds. Rarely does the frequency exceed 5/sec. On analogy with similar potentials in vertebrate muscle (Fatt & Katz, 1952) they will be called spontaneous miniature potentials (Dudel & Orkand, 1960). In Fig. 2*C* the time course of one miniature potential is

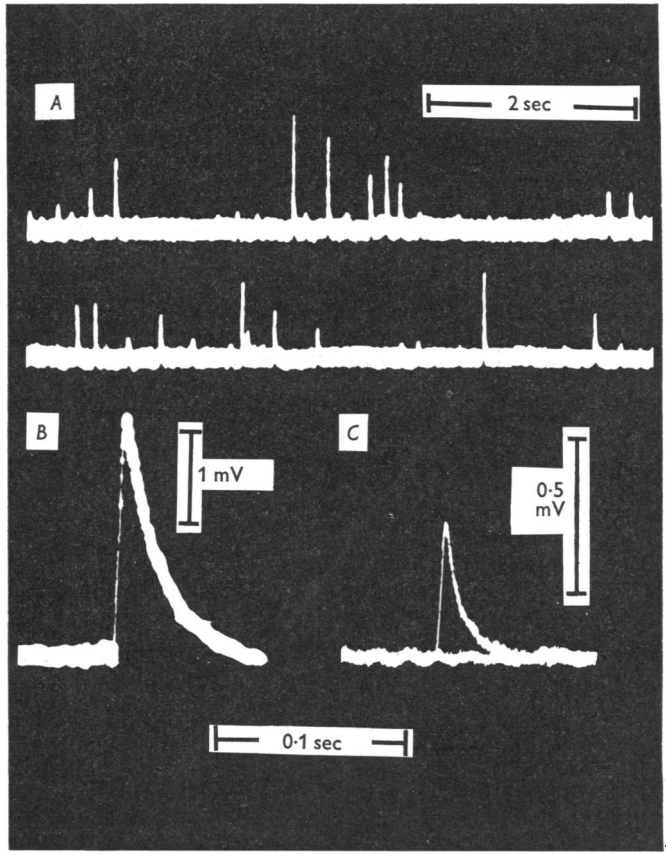

Fig. 2. *A* and *C*: Spontaneous miniature potentials recorded with an intracellular electrode from the abductor of the dactyl. Resting potential 75 mV. *B*: Excitatory junctional potential (e.j.p.), set up by single motor nerve impulse; note similar time course to spontaneous potential in *C*. Voltage calibration the same in *A* and *C*. Time calibration same in *B* and *C*.

displayed on a fast sweep speed for comparison with an excitatory junctional potential (e.j.p.) in Fig. 2*B*, set up by stimulation of the motor axon. The only difference is in peak height, the miniature potential being about 0·43 mV, whereas the e.j.p. reaches 2·5 mV.

In vertebrates the miniature potentials are confined to the end-plate regions of fibres, but in the abductor of the dactylopodite they are recorded

everywhere, as are the e.j.p.s. E.j.p.s can be recorded wherever microelectrodes are inserted into fibres, because nerve–muscle junctions occur at multiple sites over the muscle fibre surface (Wiersma, 1941; Fatt & Katz, 1953). It will be shown below that spontaneous miniature potentials originate at the same areas. Further, the spatial decrement of potentials along the muscle fibres is relatively small, so that an impressed potential change at one end does not decline by more than 40% over the entire length. Therefore, local junctional potentials are detected by an intracellular lead everywhere within muscle fibres with relatively little attenuation.

In many fibres spontaneous miniature potentials were not seen and in others they barely exceeded the noise level (20–30 μV) of our DC amplifying system. It appeared that those were large fibres, in which the input resistance, measured by a voltage drop accompanying intracellularly applied current pulses, was relatively low. The input resistance had to be between 0·2 and 0·5 MΩ for miniature potentials above 100 μV to be recorded. Accordingly, the potentials are larger in small-diameter fibres, as is also seen in vertebrates (Katz & Thesleff, 1957). This also applies to the e.j.p.s set up by motor nerve stimulation.

The following observations show the analogy between our spontaneous miniature potentials and those obtained in vertebrate junctions. (1) As in end-plates, spontaneous potentials appear at irregular intervals in a random sequence. Figure 3 gives a distribution of intervals of 240 miniature potentials. The broken line is drawn according to the equation

$$n = N\frac{\Delta t}{T}\exp\frac{-t}{T},$$

where n = the number of observed potentials at any interval,
N = number of observations,
T = mean interval,
Δt = duration of the grouping in the histogram (0·2 sec) and
t = observed interval between successive potentials (Fatt & Katz, 1952).

The fit of the observations with the curve means that there is no obvious interaction between single spontaneous potentials. (2) A little understood feature of vertebrate miniature potentials is the sensitivity with which they increase in frequency with raised osmotic pressure. This also applies to the miniature potentials in crayfish. For instance, doubling the osmotic pressure by adding sucrose has resulted in as much as a sevenfold increase in rate. (3) In vertebrate junctions it was shown that the membrane potential of the motor nerve terminals controls the average miniature frequency. This observation is important because it is probably related to

the conversion of motor nerve impulses to transmitter release. Nerve terminals in the crayfish preparation could not be depolarized by applied current. We were able, however, to depolarize the endings by excess K^+ and to get increased rates of miniature release (Liley, 1956*c*). For instance, doubling the K^+ concentrations resulted in a muscle depolarization of about 7 mV and doubled the spontaneous discharge rate. The size of the spontaneous potentials was not appreciably changed nor was the membrane resistance affected. An even larger depolarization of the muscle

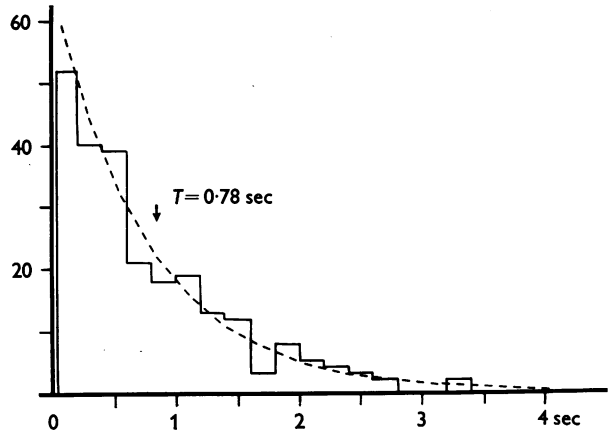

Fig. 3. Distribution of intervals between 240 spontaneous miniature potentials. Interrupted line is distribution expected for random sequence (see text). T is the mean interval between potentials.

membrane by applied current did not change spontaneous discharge rates, and thus the potassium effect could not have resulted solely from the change in muscle membrane potential. It is therefore thought that the effect was brought about by the depolarization of nerve terminals by excess K^+. (4) Another point that might relate membrane changes in the motor nerve with the discharge rate of the spontaneous miniature potentials is the transient change in their frequency following trains of excitatory potentials (cf. Dudel & Kuffler, 1961). This again resembles the behaviour of vertebrate junctions.

Fatt & Katz (1952) and del Castillo & Katz (1954) showed that in frogs miniature potentials were not set up by spontaneous conducted impulses in the motor axon. This possibility was excluded in the crayfish because the miniature potentials were not abolished by excess Ca^{2+} even if increased tenfold, or by application of β-guanidopropionic acid 10^{-4} g/ml. (Kuffler, 1960). Such treatment abolished nerve conduction and no e.j.p.s appeared with nerve stimulation. Further, extracellular recording with

micro-electrodes showed that during a single miniature potential only one active synaptic area is involved, whereas a nerve impulse activates many junctional areas (see below, Fig. 6).

There remains the unlikely possibility that spontaneous miniature potentials arise as a result of spontaneous impulse-like activity in single terminals without invasion of the axons. This also was excluded, because spontaneous miniature potentials, interspersed between e.j.p.s, did not show facilitation (Dudel & Kuffler, 1961).

Extracellular records from single junctional areas

The question now is whether the e.j.p.s set up by motor nerve impulses are built up of units that resemble the spontaneous miniature potentials. To answer this is made difficult because the innervation of the abductor fibres is distributed widely in multiple spots over the muscle surface. Intracellular leads record all the potential changes, with some attenuation, which take place across the surface membrane of the short muscle fibres. They sum or integrate the many junctional potentials and correspondingly complicate the analysis. Our question would, therefore, be better answered by recording exclusively from a single junction formed by the synaptic contact of one terminal branch.

Del Castillo & Katz (1956*a*) have shown that with extracellular micro-electrodes one can record from one 'active' spot within the vertebrate end-plate. They have discussed the recording conditions extensively and we have used this method in the crayfish. Briefly, the situation is as follows. The released transmitter causes a synaptic current to flow through the junctional membrane. The field in the low-resistance external medium is very circumscribed and only within a few micra of the small junctional membrane area will the synaptic current be dense enough to cause an appreciable voltage drop. If placed close to the junctional patch a sufficiently small extracellular electrode will record this voltage drop and thus one can obtain a relative measure of the synaptic current at an individual junctional area.

The main problem is to find such a circumscribed region. If one places a capillary electrode (0·5–2 μ opening) on the muscle surface, no e.j.p.s are seen as a rule. If, however, one conducts a tedious search along the fibre, making on the average 30–50 contacts, one usually finds a spot where potentials appear with nerve stimulation. These spots are sharply localized and the potentials disappear with a sideways movement of the electrode tip of several micra.

In Fig. 4 the motor nerve was stimulated at 5/sec. The upper line shows the e.j.p.s recorded intracellularly. The lower line presents the simultaneous e.j.p.s measured extracellularly at a single junctional area. The

e.j.p.s from the fibre interior are relatively large and show fluctuations in height that are usual at such low frequencies. The intracellular potentials are positive, whereas the surface micro-electrode records negative potentials. The most interesting aspect is the relationship of potential fluctuations in the two records. Under the extracellular micro-electrode the single synaptic area did not give any response with one of the nerve stimuli (arrow). At that time, however, the intracellular electrode happened to record the largest e.j.p. in the series. With this particular nerve stimulus many of the junctions, but not the one under the extracellular lead, must have been activated. Also, in the other records there is no obvious correlation between the intracellular potential size and the extracellular one. More detail of time relations is shown in Fig. 5 where the sweep speed is rapid.

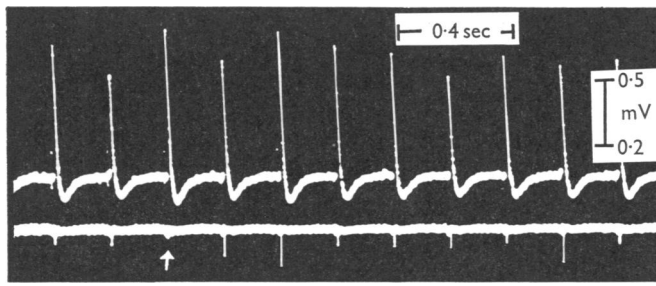

Fig. 4. Upper line: intracellular recording of e.j.p.s at stimulation rate of 5/sec. Resting potential 82 mV. Lower line: simultaneous records from a single junctional area with an extracellular microelectrode. Arrow marks failure of transmission in single junction; note independence of fluctuations in intra- and extracellular records. 0·2 mV refers to amplification in lower beam. Diphasic component of intracellular records is due to AC amplifier.

Five records, taken consecutively during stimulation at a rate of 2/sec, are superimposed. The intracellular e.j.p. sizes in the upper record fluctuated very little, but in comparison the potentials at the single junctional area varied over a wide range. Two nerve impulses failed altogether to set up an extracellular e.j.p. Preceding the extracellular junctional potentials by about 1 msec, there occurred regularly another phasic potential which represents the nerve impulse (arrow) arriving in the vicinity of the junction. A neuromuscular delay, thus measured, of about 1 msec was seen regularly. The intra- and extracellular e.j.p.s had practically the same latent period; the duration of the extracellular potentials, however, did not last much longer than the rising phase of the intracellular e.j.p.s. Apparently the 'active' phase, during which the transmitter causes synaptic current to flow, is largely confined to the rising phase of the e.j.p. Following this period, the potential decline of the intracellular e.j.p.s is passive, i.e. determined by the muscle time constant. The current that

flows passively is distributed over the whole membrane area. The membrane current density is therefore low and the resulting extracellular potential drop is too small to be measured.

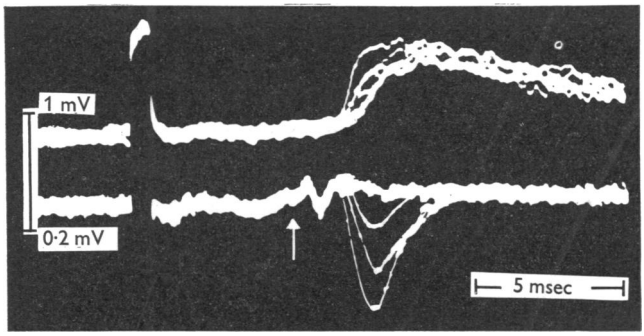

Fig. 5. Upper line: intracellular recording of e.j.p.s at stimulation rate of 2/sec, five sweeps superimposed. Lower line: simultaneous extracellular e.j.p.s from a single junctional area recorded with micro-electrode. Arrow marks beginning of motor nerve impulse near junctional area. Latent period of intra- and extracellular e.j.p.s is same but time course of extracellular e.j.p.s is shorter and their range of fluctuation is much greater. Two of the five stimuli fail to set up e.j.p.s in extracellular record.

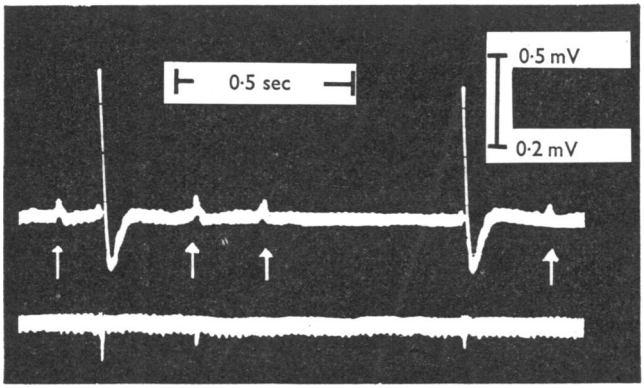

Fig. 6. Motor nerve stimulated at 1/sec. Upper line: intracellular record of two e.j.p.s and of four spontaneous miniature potentials (arrows). Lower line: simultaneous extracellular record from a single junctional area. Only one spontaneous potential occurs (simultaneous with second arrow). It is of the same amplitude as the second e.j.p. set up by a nerve impulse. 0·2 mV refers to lower beam amplification.

In addition to e.j.p.s one could record spontaneous potentials if the extracellular electrode was placed at an 'active' spot. An example is given in Fig. 6. The nerve was stimulated at 1/sec and two extra- and intracellular e.j.p.s are illustrated. Intracellularly, four spontaneous

miniature potentials are also seen (arrows). The second one is associated with an extracellular spontaneous potential. When such a potential occurred it was always accompanied by an intracellular spontaneous miniature potential. Such extracellular potentials were seen only about once a minute, as compared with intracellular ones which occurred about once a second. They were found exclusively at the active spots where the localized extracellular e.j.p.s were recorded. With a small sideways shift of the electrode, both potential types disappeared. Spontaneous extracellular potentials had a size similar to that of the smallest e.j.p.s set up

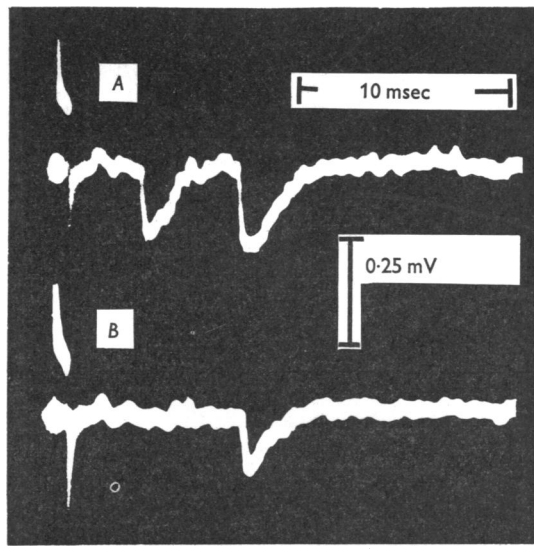

Fig. 7. Extracellular records from single junctional area during motor nerve stimulation. *A*. Second potential is e.j.p. set up by nerve impulse; first is a spontaneous miniature potential. *B*. e.j.p. set up by motor nerve impulse as in *A*. Note similar size and time course of e.j.p.s and of the spontaneous potential.

by nerve stimulation and recorded at the same spot. An example is contained in Fig. 6 (second e.j.p.). Not only sizes, but also the time courses of the smallest extracellular e.j.p.s and of spontaneous potentials, were practically identical. In Fig. 7*A* a spontaneous potential, by chance, fell just before an e.j.p. taken at a fast sweep. The two e.j.p.s (Fig. 7*A* and *B*) set up by nerve impulses indicate the range of fluctuation of this response.

One can conclude that the intra- and extracellular spontaneous potentials represent the same phenomenon. Like the e.j.p.s, they arise at neuromuscular junctional areas. The fact that many more intracellular than extracellular spontaneous potentials are observed means that spontaneous potentials arise at numerous terminal regions. The ratio of about

1:60 of the frequency of extracellular to intracellular spontaneous potentials may be a rough measure of the number of synaptic contact areas in a muscle fibre. The striking similarity of the spontaneous extracellular potential and the smallest e.j.p. set up by nerve impulses leads one to suspect that, as in the frog end-plate, the spontaneous potentials represent the same units as those released normally in great numbers upon nerve stimulation (see later).

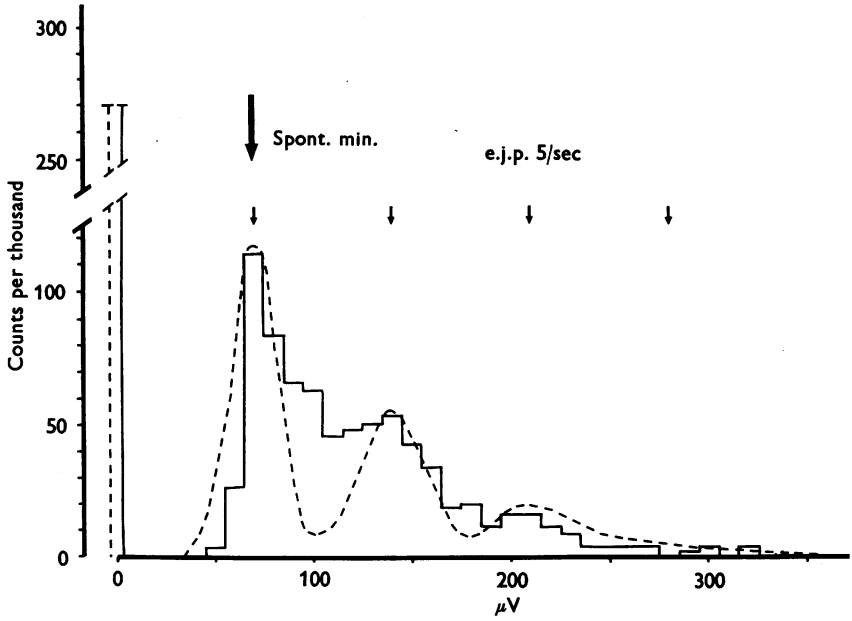

Fig. 8. Histogram of size distribution of extracellularly recorded e.j.p.s from a single junctional area. Ordinate scaled to 1000 counts. There are 270 zero potentials, i.e. failures of transmission. Broken line is drawn according to Poisson's theorem for a quantum size of $E_1 = 70$ μV with a standard deviation $\sigma_1 = 12$ μV. The quantum content per stimulus is $m = 1{\cdot}3$. Small arrows indicate the multiples of the unit size. Big arrow gives the average size of the spontaneous miniature potentials.

Statistical treatment of extracellular e.j.p.s from single synaptic areas

The occurrence of spontaneous miniature potentials and the striking fluctuations in the size of the e.j.p.s recorded extracellularly (Figs. 4 and 5) suggest an analogy with the situation at the vertebrate neuromuscular junction, i.e. that the crustacean e.j.p. is also built up of units representing quantal release of transmitter. In order to test the applicability of this idea more closely, a statistical analysis of the type used by del Castillo & Katz (1954), Boyd & Martin (1956) and Liley (1956*b*) was needed. We recorded from an extracellular junctional area the responses to 1000 nerve

stimuli given at a rate of 5/sec. The graph of Fig. 8 is a histogram of the recorded e.j.p. sizes. The distribution does not fall symmetrically around a mean but has a complicated form. It contains 270 misses, i.e. occasions when an impulse failed to set up any extracellular junctional potentials.

If the potentials are made up of unit responses one expects that Poisson's theorem should describe the distribution. In the context of the present problem this theorem applies if there exists a very large pool of possibly reacting units and if the probability that any one particular unit should be released on a single nerve stimulus is very low. The theorem predicts the probability n_x/n (n number of stimuli) that a nerve stimulus will cause the release of no unit ($x = 0$), one unit ($x = 1$), two units ($x = 2$) or any other multiple. It states that n_x/n depends only on the average number m of units released by a nerve stimulus. The equation is:

$$\frac{n_x}{n} = \frac{m^x}{x!}\,\mathrm{e}^{-m} \tag{1}$$

A first test of our assumption, that the e.j.p.s are composed of quanta, will be that a Poisson distribution can be found that fits the observed e.j.p. size distribution in Fig. 8. The information gained from spontaneous miniature potentials will not be used for the construction of the Poisson curve, because the extracellular spontaneous potentials are infrequent. They will, however, be used afterwards as an independent control. If equation (1) is written for consecutive values of x, a simple series of equations results which is convenient for the construction of a Poisson curve that fits:

$$n_0 = n\mathrm{e}^{-m}, \tag{2a}$$

$$n_1 = m n_0, \tag{2b}$$

$$n_2 = (\tfrac{1}{2}m)n_1, \tag{2c}$$

$$n_3 = (\tfrac{1}{3}m)n_2. \tag{2d}$$

It is obvious from this series that if n_0 is known, m can be calculated from equation (2a), and then all the values n_1, n_2, n_3, ... follow.

Experimentally n_0 can be found by merely counting the number of failures. Thus this value is a convenient starting point for the construction of a Poisson curve which fits the histogram of Fig. 8. According to equation (2a) for $n_0/n = 270/1000$ we calculate an average number of units released per stimulus of $m = 1{\cdot}3$. And the equations (2) further predict the number n_1, n_2, n_3, ... of potentials of single and multiple quantum size. These numbers now have to be converted into unit potentials. The size of the average unit potential E_1 can be obtained from the relation

$$E_1 = \frac{\bar{E}}{m}, \tag{3}$$

where $\bar{E}$ is the average size of the extracellular e.j.p. which can be measured.

For the experiment of Fig. 8, E_1 was 67 μV. The unit quantum is not strictly of the same size each time it is recorded. E_1 is an average unit potential with a statistical fluctuation around a mean value. For instance, the number of single units n_1 represents the area under a Gaussian curve (normal distribution) of a certain variance σ_1 around the unit size E_1. As a consequence the appropriate Poisson curve for Fig. 8 is obtained by finding σ_1, by trial and error, and adding the Gaussian curves with the areas n_1, n_2, n_3,... around the mean values E_1, E_2, E_3,.... The procedure is illustrated in Boyd & Martin (1956). It is seen in Fig. 8 that the general form of the Poisson curve agrees with the experimental values.

A control and confirmation of the validity of calculating unit sizes comes from measuring the average size of the spontaneous potentials. The spontaneous miniatures should be of unit size, because it is very improbable that two or more units should be released spontaneously at the same time. During the experiment of Fig. 8, ten extracellular spontaneous miniature potentials were observed. Their average size was 71 μV with a variance of 10 μV. This agrees very well with the size of the unit quantum E_1 calculated for the Poisson curve in Fig. 8. It can thus be concluded that the e.j.p.s are composed of units of the same average size as the spontaneous potentials.

In the above statistical treatments of the extracellular e.j.p.s we calculated the unit size from the relative number of failures and the average e.j.p. size. Then we compared this value with the average size of the spontaneous potentials. In the statistical papers on the vertebrate end-plate the converse procedure was used by del Castillo & Katz (1954), Boyd & Martin (1956) and Liley (1956*a*). They determined first the average spontaneous potential size and used it as the unit quantum for the construction of a Poisson curve, which had to fit the observed distribution of end-plate potential sizes. This approach was not used in this study because the extracellular spontaneous potentials are too infrequent in the crayfish junctions for the average size of spontaneous potentials to be accurately determined.

Equation (2*a*) provides an easy way of determining quantum size and quantum content in different junctional areas. Some examples are given in Table 1; the unit size E_1 has been calculated there from the relative number of failures n_0/n and the average potential $\bar{E}$. As expected, in all the samples the values for E_1 agree with the values found for the average spontaneous potential. These examples add further weight to the above conclusion that the e.j.p.s are indeed quantal responses. Further supporting examples, as in Table 1, are given in the following papers.

DISCUSSION

The following picture of crayfish neuromuscular transmission has emerged from the present study. There are discrete junctional areas which occupy a small portion of the muscle fibre surface, as seen from the fact that a prolonged search is necessary to locate them. In these spots synaptic

currents flow after nerve stimulation and it is these currents that have been shown to be quantal in nature. We assume that the quanta of current represent quanta, or units, of transmitter released from nerve terminals. At the same spots there occur spontaneous miniature potentials which are of unit quantum size. They are not released by spontaneous impulse activity in the axons or in the nerve terminals (see also Dudel & Kuffler, 1961, p. 536).

In all aspects covered by this study there was a rather complete analogy between mechanisms of transmission in vertebrates (del Castillo & Katz, 1956*b*) and in crayfish. A quantitative difference in the transmission process is the much lower probability of release of quanta in individual junctional areas of the crayfish. Our preparation in a 'normal' solution works like the nerve–muscle junction of twitch fibres in the frog, where

TABLE 1

Expt. no.	Stimulus frequency	n	m	$\bar{E}(\mu V)$	$E_1(\mu V)$	$E_s(\mu V)$
1	5/sec	121	2·2	155	70	70
2	5/sec	961	1·32	85	65	69
3	2/sec	243	0·27	18	66	65
4	1/sec	115	0·31	15	49	48

n = number of stimuli; m = average quantum content of extracellular e.j.p. determined from $m = \log_e n/n_0$; n_0 = number of transmission failures; $\bar{E}$ = average size of extracellular e.j.p.; E_1 = size of quantum determined from $E_1 = \bar{E}/m$; E_s = average size of extracellular spontaneous potentials.

the probability of release was depressed by excess Mg^{2+} and/or decreased Ca^{2+} (del Castillo & Katz, 1954). Spontaneous potentials of low frequency were seen by Burke (1957) in the frog's slow muscle fibres.

Another interesting difference is the shorter duration of synaptic current flow in the crayfish. The current barely outlasts the e.j.p. rising phase, whereas in the frog there is considerable current flow during the first half of the falling phase (del Castillo & Katz, 1956*a*; Liley 1956*a*; Takeuchi & Takeuchi, 1959).

A weak point in our line of conclusions about the transmission mechanism lies in our having measured quantal synaptic currents only. We interpret these quanta of currents as caused by packets of transmitter. We do not have direct evidence for this view. There is a possibility that the transmitter itself is not released in packets but triggers a quantal response in the post-synaptic membrane. We consider this possibility to be very unlikely, mainly because of the complete analogy that exists with the vertebrate junction. There this alternative was excluded because the transmitter is known. The size of the quanta could be graded by changing the amount of transmitter reaching the post-synaptic receptor sites by the use of applied ACh, curare, or of inhibitors of cholinesterase. These sub-

stances, however, did not affect the number of released units. In our preparation these experiments cannot be done because the transmitter is not known.

The extension of the quantal release mechanism of transmission from frog and mammalian neuromuscular junctions (del Castillo & Katz, 1956*b*); Boyd & Martin, 1956; Liley, 1956*a*) to crayfish makes one suspect that this system may be a much more general feature of synapses. In particular, the quantal mechanism is not confined to the cholinergic systems because in crayfish junctions ACh is not a transmitter. In addition, the quantal mechanism does not seem to be confined to a specific anatomical synaptic structure. The mammalian neuromuscular junction has a compact terminal configuration, being much more confined than in the frog, where the nerve splits up into a fine terminal arborization which may extend over several hundred micra. (For a survey, see Couteau & Taxi, 1952.) The innervation in the frog's slow fibres is also spread over most of the surface, as in the crayfish (Kuffler & Vaughan Williams, 1953), and spontaneous miniature potentials have been demonstrated there (Burke, 1957). In the crayfish abductor of the dactyl the widespread innervation formed by one motor fibre may be regarded as an extended 'end-plate'. The fine detail of the junctions could not be resolved by the light microscope. Another different feature of the muscles is the inhibitory innervation which, as far as can be seen, runs together with the motor fibre (Biedermann, 1887; Mangold, 1905; Hoffmann, 1914; D'Ancona, 1923; Tiegs, 1924; van Harreveld, 1939).

The statistical treatment of the excitatory potential, showing its quantal composition, was found useful in this study for linking the spontaneously released miniature potentials to excitatory transmission. This quantal analysis of the e.j.p. gains further significance in the following papers; it was found that the unit sizes remain constant during drastic changes of the e.j.p. brought about by the physiological mechanisms of facilitation and inhibition.

SUMMARY

1. The abductor of the dactylopodite in the crayfish is supplied by only one motor axon. Over the surface of each muscle fibre there are distributed numerous individual junctional areas. Intracellular electrodes record practically all excitatory junctional potentials (e.j.p.s) of a muscle fibre. Extracellular capillary electrodes record, selectively, the strictly localized potentials of individual junctional areas.

2. The e.j.p.s at individual junctional areas are 'quantal'. They are composed of discrete units released with a certain probability by a motor nerve impulse. This conclusion is based mainly on the finding that the distribution of sizes of e.j.p.s can be described by Poisson's theorem. The

probability of release of quanta at individual junctional areas is low, and frequently a nerve impulse fails to release a single quantum. The e.j.p. of the whole muscle fibre is thus the sum of all the quantal contributions from distributed single junctional areas.

3. In addition to e.j.p.s, spontaneous miniature potentials can be recorded. They arise at the same individual areas and have the same size and time course as the units released by the nerve impulse. Spontaneous miniature potentials recorded intracellularly recur in a random sequence at rates around 1/sec. Recorded extracellularly at single junctional areas they are seen about once a minute. Their frequency but not their size is influenced by repetitive motor nerve stimulation, raised osmotic pressure and moderate depolarization by excess K^+. Spontaneous miniature potentials are not due to spontaneous nerve impulses, because they are not affected when nerve conduction is blocked by high Ca^{2+} or by β-guanidobutyric acid.

4. The quantal nature of transmission in the crayfish is similar to that at vertebrate neuromuscular junctions. It is concluded that the quantal e.j.p.s are set up by the release of packets of transmitter from motor nerve endings.

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