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Mechanisms of Synaptic Transmission

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INTRODUCTION

THE propagation of an impulse along a nerve or muscle fiber is brought about by two coupled processes: (i) cable transmission, which allows an electric potential change to spread along a short distance, but with rapid attenuation, and (ii) a boosting mechanism by which the full signal strength is regenerated at each point. If either of these processes is interfered with, the signal will be blocked and will fade out locally. The cable mechanism depends upon the continuity of the fiber structure, with a relatively low-resistance core and high-impedance surface layer. During the impulse, sufficient current must be able to flow forward along the inside of the axon and outward through the resting membrane to stimulate it. If one were to close the core with a high-resistant transverse membrane, or to place a low-resistance shunt across the fiber surface, transmission would be impaired and probably would fail at that point. The very reason why thousands of axons packed together within one nerve bundle can conduct their messages independently, without mutual interference, rests on the absence of structural continuity, and so of an effective cable connection between them.

The purpose of this paper is to consider what happens at "synapses," the points of contact between one nerve cell and the next, or between nerve and muscle fiber. There is no sign of cytoplasmic continuity between the different cell units. Electron-microscope evidence shows that the membranes of the synapsing cells are arranged in close proximity, though in general they do not seem to fuse or to come into intimate contact. The electron micrographs, however, do not reveal anything about the electrical properties of the contacting surfaces; and one has no means of guessing intuitively whether or not an effective cable linkage exists across the synapse.

ELECTRICAL AND CHEMICAL TRANSMISSION

There are, in principle, two basically different modes of synaptic transmission, electrical and chemical. *Electric* transmission implies that in spite of apparent morphological complexities, an effective local-circuit connection exists which allows sufficient current to pass from one cell to the next to restimulate it. In other words, the transmission is one continuous process without any essential change at the synapse. There must, of course, be *some* difference; for all synapses have the property of functioning only in one direction unlike nerve fibers which can conduct impulses with equal facility in either direction despite the fact that in normal life, because of their terminal synaptic connections, they are used for one-way traffic only.

Chemical transmission implies the intervention of an entirely different process specific for the synaptic area. It presupposes that the ordinary cable-connection is interrupted at the contact point between the cells, and replaced by the agency of a chemical mediator. A specific chemical stimulant which is synthetized and stored inside the nerve terminal is liberated by the nerve impulse. When the substance is released, it attaches itself to special receptor molecules in the surface of the contacting postsynaptic (or effector) cell. This chemical combination leads to a membrane change which gives rise to a local depolarization of the effector cell. When the depolarization exceeds the threshold level, a new action potential is set up which then travels along the cell in the manner previously described. Thus one has, interposed between two separate waves of propagated electric activity, a secretion of a specific substance from one cell, and a chemoreceptor reaction in the surface of the next cell.

Now, it is not possible to predict without thorough experimental examination which of these two modes of transmission occurs at a particular type of synapse. Various attempts have been made to generalize by drawing analogies from the few cases which have been explored; and in recent years the view has become prevalent that transmission is llkely to be chemical at all synapses, but that a variety of substances are being employed in different cases, and only a few of them, like acetycholine and noradrenaline, have so far been identified. This view, however, seems a little too sweeping, and the discussion is begun, therefore, by quoting an example of electric transmission which has just been brought to light by the work of Furshpan and Potter.[1] This may well be an exception to the rule, but it provides a definite warning against too much generalization in this field.

Furshpan and Potter used a "giant" synapse in an abdominal ganglion of the crayfish cord. This is a contact point between a very large nerve fiber which runs through the central nervous system of the crayfish and a somewhat smaller motor axon which emerges from the cord to supply the flexor muscles of the "tail." This synapse was chosen for two reasons: (a) because the large size of the two contacting cells made it possible for a pair of microelectrodes (one to pass current and one to measure membrane potential) to be introduced into each of them; (b) it seemed *a priori* that electric transmission might be feasible at this synapse, more so than at other types where minute nerve endings usually

terminate in contact with a huge cell (which implies, from the electrical point of view, a poor mismatch, very little current being available from the high-impedance terminals to discharge the large surface of the postsynaptic cell). Furshpan and Potter were able to show that the membrane contact of this special synapse acts as a good electric rectifier, allowing current to pass relatively easily from the presynaptic to the postsynaptic cell, but not in the reverse direction. In other words, at this particular synapse, an adequate cable connection exists between the interior of the two cells, in the *normal* direction of impulse travel alone. Provided the internal potential of the prefiber was *higher* than that of the postfiber, electric current could flow across and influence the membrane potential of the adjoining cell. The result is that a depolarization such as occurs during the impulse can be transmitted in the normal *orthodromic* direction, but not the other way. And, conversely, a local hyperpolarization, produced experimentally by passing a current inward through the fiber membrane was found to be transmitted only in the *antidromic* direction (from postfiber to prefiber) but not the other way. Thus, at the giant synapse of the crayfish, one has a case—so far, the only known example—of electric transmission, in which the action current generated by the arrival of an impulse in the presynaptic cell is passed on without finite delay and can directly depolarize and thereby excite the postfiber. One-way transmission is owing to the valve-like one-way resistance of the synaptic contact membranes.

There are only a few giant synapses available in nature allowing such a direct experimental approach to both sides of the junctional region. In most cases, the presynaptic nerve endings are too small to be tackled with intracellular electrodes and their electrical behavior has to be inferred from a more indirect approach. It is of great interest, however, that at another giant synapse, in the stellate ganglion of the squid, Bullock and Tasaki and Hagiwara[2,3] obtained evidence of a different kind; they observed a definite local delay in the propagation of the electrical change, indicating a stoppage of the local-circuit transmission at the junction; there was no detectable transfer of subthreshold cable signals in either direction. These observations provide another fair warning against attempts to generalize about synaptic mechanisms.

If one now takes an entirely different case—namely, the skeletal neuromuscular junction—one finds here one of the few examples of a synapse where chemical transmission has been firmly established. It was shown by Dale and his colleagues that a specific cholinester, almost certainly identical with acetylcholine, is released from the active motor-nerve endings. This substance is a very potent local stimulant and, provided it is applied rapidly to the junctional end-plate region of a muscle fiber, causes a local depolarization of the cell membrane and sets up propagated impulses and contraction in the fiber. The chemical effect is localized to the synaptic area of the muscle surface; it is at these points exclusively that a number of chemical blocking agents, like curare, act (apparently by a competitive attachment to the acetylcholine receptors). By histochemical methods, a high local concentration of a specific enzyme, acetylcholinesterase, has been found at the same point; the apparent purpose is to hydrolyze the transmitter substance within a very short time after it has exerted its action. Electrical studies have shown that there is an irreducible delay of 0.5 to 1 msec between the arriving nerve impulse and the start of the so-called end-plate potential (which is the local depolarization in the muscle fiber produced by the transmitter substance). There is no cable transfer of electric current, of either polarity, directly from the nerve axon to the muscle fiber. When potential changes are imposed on the terminal portion of the motor nerve, these changes do not spread beyond the nerve terminal, but can be shown to increase the rate at which acetylcholine is being released from the nerve endings and so, indirectly, to influence the membrane potential of the muscle fiber.

The most direct way of establishing chemical transmission by nerve impulses would be to show that a substance is released, on nerve stimulation, into the circulating fluid, and when applied to a remote effector cell produces the same, excitatory or inhibitory, action. This has been achieved in a few cases, notably the classical experiment of Otto Loewi in which he demonstrated the role of acetylcholine as the transmitter of nervous inhibition of the heart beat. Usually, such a direct demonstration is not feasible because of the enormous dilution of the transmitter agent, on the way from its primary point of release and action to the assaying tissue. Indeed, this discrepancy between (i) the amount of acetylcholine (ACh) which has to be applied artificially to stimulate a muscle, and (ii) the much smaller quantities which are released into the perfusion fluid from stimulated nerve endings, has been used as an argument against the validity of the chemical-transmitter theory. By a method of microscopic ionophoresis, much more effective applications have been made recently, and it has been possible to show that as little as 10^{-16} g equiv of ACh can give rise to an effective, superthreshold, depolarization of the muscle fiber. This is still a few hundred times more than the amount per impulse recovered in the earlier perfusion experiments, but it must be remembered that even with the best micropipettes one cannot reduce the average diffusion distance to that of the natural synaptic contact. The remaining quantitative discrepancy is entirely within the range to be expected and hardly can be used as a theoretical counterargument. The ionophoretic microtechnique has shown a number of other interesting results; it has confirmed the extremely critical localization of the chemoreceptors at and within

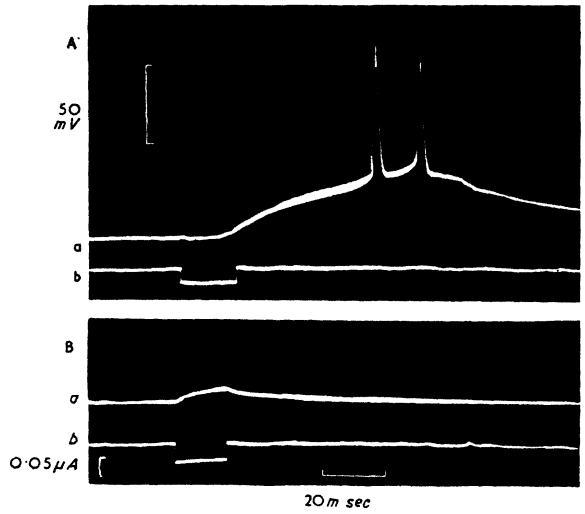

FIG. 1. External and intracellular application of acetylcholine to a motor end plate [from J. del Castillo and B. Katz, J. Physiol. **128**, 157 (1955)]. Intracellular recording of membrane-potential changes from the junctional region of a frog muscle fiber. In *A*, an ACh-filled micropipette was placed on the outside of the end plate, and a quantity of ACh was released by passing a brief outward-directed current pulse through the pipette (registered in *b*). It produced the effect shown in trace *a*: a depolarization developing after a diffusion delay and culminating in two spikes. Between records *A* and *B*, the ACh-pipette entered the muscle fiber. An outward pulse produces now a small, direct potential change, owing to the passage of current through the fiber membrane. (If, for comparison, a KCl-filled micropipette is used, no potential change is produced by the pulse until the pipette has entered the fiber, when the effect is identical with that recorded in *B*, a.)

the neuromuscular junction. Moving the tip of the pipette by several microns can substantially reduce the effect of a given dose. Furthermore, it has been possible (Fig. 1) to insert the tip into the interior of the muscle cell, and so apply the acetylcholine alternatively to the external and internal side of the postsynaptic end-plate membrane.[4] The result showed that a depolarization was produced only by external, not by intracellular application; and this was observed with acetylcholine as well as carbamylcholine (a substance of similar action, but not destroyed by the local cholinesterase), and the same result was found for the blocking action of curarine. It seems that the first chemical attachment on to the receptor molecules must take place at the external surface of the end-plate membrane, which is, of course, the side facing the nerve endings from which the acetylcholine emerges under normal conditions.

Before discussing other peculiarities of the neuromuscular junction, the principal features and problems inherent in chemical transmission in general may be considered briefly.

There are two main steps interposed between the arrival of a presynaptic and the departure of a postsynaptic impulse: (i) the process by which the arriving impulse releases the transmitter substance, from its storage place inside the terminal into the narrow cleft between the contacting cells—this is a special case of what has been called "neuro-secretion"; (ii) there is the process by which the transmitter substance becomes attached to specific molecules in the postsynaptic cell surface and causes its electric membrane properties to change—this is a special example of *chemoreceptor* action, that is a process analogous to that occurring in our various chemical sense organs where a minute concentration change of some specific substance is registered in the form of sensory nerve impulses. As an intermediate step, one should consider also the mechanism by which transmitter molecules are transported across the small synaptic gap; however, the path length is only a fraction of a micron, and the time taken up by simple diffusion over such a short distance is well within the range of the observed synaptic latency.

EXCITATORY AND INHIBITORY CHEMORECEPTOR ACTION

To begin with, consider the second step, that is, the chemoreceptive mechanism. How do transmitter substances alter the membrane potential? Only a very incomplete answer to this question can be given. In general, the primary action leads to the opening of some ionic permeability channel in the membrane. Depending upon the size or specificity of this ionic channel, the membrane potential either tends to *fall* toward a low level, well beyond the firing threshold of an impulse, or it may become stabilized in the vicinity of the resting level or even tend to rise somewhat (hyperpolarize). In the first case, excitation ensues; in the last cases, one obtains an opposite, inhibitory, action (see Eccles[5]). But it may be noted that, underlying all of these changes, there is a common primary effect—namely, an increase of some ionic conductance.

For example, at the motor end plate there is evidence that acetylcholine causes the membrane permeability to increase, simultaneously, to several monovalent cations (e.g., sodium, potassium, ammonium) and possibly opens up an indiscriminate aqueous channel to all small ions on either side of the membrane. The result is a depolarization which has a "null point" at about 10 to 20 mv, negative inside, which corresponds to the level of a free-diffusion or liquid-junction potential between cytoplasm and external fluid. The effect is to short-circuit and depolarize the surrounding muscle membrane beyond the level at which a new impulse arises which then travels rapidly along the whole length of the muscle fiber. The methods by which these conclusions were reached have been described elsewhere;[6,7] briefly, they consisted in measuring the current/voltage relation across the end-plate membrane, and observing the particular level of the membrane potential at which the electromotive effects of ACh reversed, with normal as well as with altered composition of the ionic environment.

While ACh has a depolarizing and excitatory effect at the end plate, it produces the opposite action, that is

hyperpolarization or stabilization of the resting potential, in the regions of the heart muscle onto which it is released by impulses in the vagus nerve. Here also, the basic effect is an increase of ionic conductance, but the channel which is being opened is restricted to potassium, and does not include sodium. As a result the membrane tends to move towards, or to be held at, the potassium equilibrium potential which is usually somewhat greater than the existing resting potential (hence, a hyperpolarization).

Somewhat similar changes appear to be associated with the excitatory and inhibitory synapses in the motor neurons of the spinal cord.[5] At these junctions, the transmitter substances are unknown, but the "null points" of the potential changes which they produce have been determined and correlated with the existing ionic concentration gradients. Here also, an inhibitory hyperpolarization occurs which appears to be associated with an increase of membrane conductance to various small ions but excluding sodium; while excitation apparently arises from an indiscriminate "short circuit" in which sodium as well as the other small ions are allowed to pass.

Although the transmitter effects are fairly well understood in terms of ionic conductance changes, and the subsequent steps leading to excitation or inhibition present no special problem, the molecular mechanism by which the chemical attachment, e.g., of acetylcholine, to the receptive sites of the membrane alters its permeability is still far from understood. At one time it was thought possible that ACh^+ ions might produce a local depolarization without permeability change, simply by being able to move very rapidly through specific channels into the interior of the muscle fiber. This idea had to be abandoned when it became clear that the transfer of Coulombs during the end-plate depolarization exceeds the charge on the available ACh ions by several orders of magnitude. The observation shown in Fig. 1—namely, that a positive quantity of charge applied directly into the interior of the cell is much less effective in depolarizing the fiber membrane than an equivalent charge of ACh ions applied on the outside—illustrates this point rather clearly: most of the externally released ACh ions will diffuse away and have no chance of penetrating, or even colliding with, the end-plate surface. Yet under its influence much more positive charge enters the fiber than with the direct intracellular discharge. The conclusion is that the relatively few ACh ions released from the nerve terminal cause a vastly greater quantity of other, ambient, ions to flow through the end-plate membrane, and so achieve the great amplification of local current which is needed to transmit an impulse from the minute nerve endings to the much larger muscle cell.

Regarding the molecular combination between ACh and receptors and the subsequent chain of events, all that can be said at present is that a study of various chemical inhibitors (e.g., del Castillo and Katz[8]) suggests the presence of a 2- or 3-stage process whose kinetics resemble those of many enzyme-substrate reactions. Substances like tubo-curarine appear to act as competitive inhibitors, by interfering with the initial site of attachment, without themselves leading to the next phase which involves a change in the physical membrane properties.

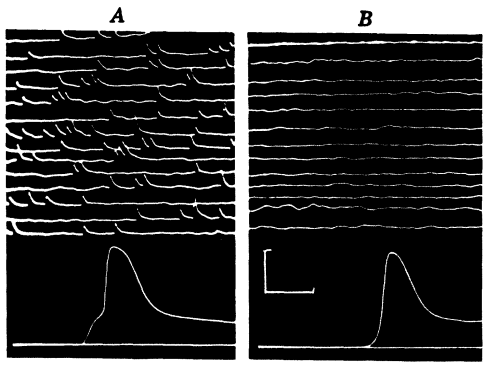

FIG. 2. Spontaneous miniature end-plate potentials. *A:* A recording microelectrode was placed inside a frog muscle fiber at the nerve-muscle junction. *B:* The electrode was placed 2 mm away into the same muscle fiber. The upper portions were recorded with slow speed and high amplification and show the occurrence of spontaneous small potential changes, restricted to the junctional region (calibrations: 3.6 mv and 47 msec). The lower portions show the response to a nerve impulse with fast-speed and low-gain recording (calibrations: 50 mv and 2 msec). The stimulus was applied to the nerve at the beginning of the trace; response *A* (at the end plate) shows the step-like initial end-plate potential which leads up to the propagating wave; response *B* shows only the propagated action potential, delayed by conduction over a distance of 2 mm [from P. Fatt and B. Katz, J. Physiol. **117**, 109 (1952)].

Quantal Nature of Acetylcholine Release

An interesting feature which has emerged during a detailed study of the vertebrate nerve-muscle junction is that the release of ACh from the motor nerve terminals occurs in discrete packets or quanta each containing a large number of molecules. Even in the absence of a nerve impulse, such packets are released "spontaneously" at infrequent random intervals (Fig. 2). The arrival of an impulse at a cell junction apparently causes a few hundred events to be synchronized within a fraction of a millisecond instead of going on at a leisurely average rate of about 1/sec.[9]

The evidence for this state of affairs was obtained soon after it became possible to apply intracellular recording electrodes to the motor end plate. If a recording electrode is inserted into a resting muscle fiber well away from its junctional region, one observes a steady resting potential of about 90 mv, negative inside. But as one approaches the end plate with the recording probe, a characteristic form of spontaneous activity shows up which consists of an intermittent random

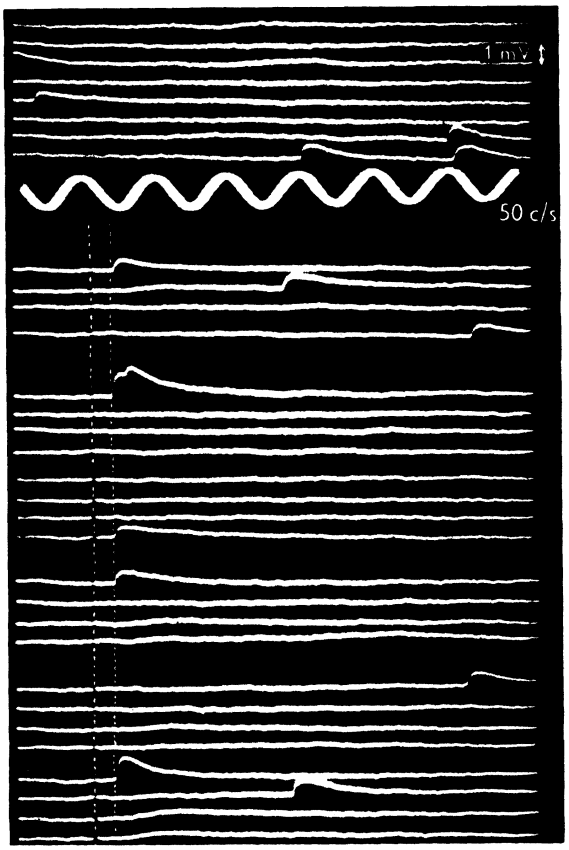

FIG. 3. Quantal units of end-plate response, recorded intracellularly from a muscle fiber in a calcium-deficient and magnesium-rich medium. The top portion shows a few spontaneous potentials. The lower part (below the 50-cps time signal) shows, in addition, the responses to single nerve impulses. Stimulus and response latency are indicated by a pair of dotted lines. There was a high proportion of failures, and only 5 single unit-responses to twenty-four impulses [from J. del Castillo and B. Katz, J. Physiol. **124**, 560 (1954)].

discharge of minute potential changes of standard size and time course. Each deflection is a transient depolarization of the order of 0.5 mv, with a rapid (1 msec) rise and a slower decay, lasting altogether about 20 msec. It resembles in many respects the end-plate potential (e.p.p.)—that is, the immediate depolarization of the end plate produced after the arrival of a nerve impulse, but differs from it in its much smaller size (about 1%) and its spontaneous random occurrence. Fatt and I called it the miniature end-plate potential. It resembles the e.p.p. in its time course, its restricted localization to the innervated region of the muscle fiber, and its pharmacological reactions. It is reduced in size by curare, and its amplitude and duration increases when the local hydrolysis of ACh is prevented by a potent anti-esterase. In both respects, the miniature potential behaves exactly like the depolarizations produced by an applied dose of ACh, and we believe, therefore, that the spontaneous discharges arise from local random impacts of ACh on to the end-plate receptors. The source of these impacts is evidently a spontaneous release or leakage of ACh from the motor-nerve terminal where the substance is stored, for the miniature potentials vanish in the course of experimental nerve degeneration, at a time when neuromuscular transmission fails.

We considered the possibility that random molecular diffusion of ACh from the motor-nerve ending might be responsible for the minature e.p.p.'s. If this were true, then the same type of discharge ought to be elicited, at vastly increased frequency, by applying ACh to the fluid surrounding the end plate. This, however, is not the case: the depolarization which one then observes is continuously graded in size and time course, depending upon the dose and length of the diffusion path. It is clear that the effects of single molecular impacts of ACh must be far below the resolving power of our re-

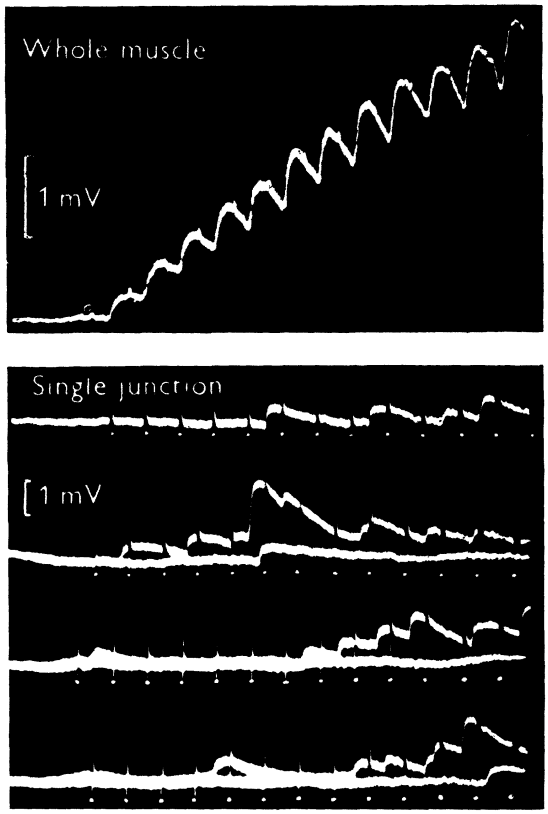

FIG. 4. Statistical properties of the end-plate response. The nerve-muscle preparations were blocked by a high magnesium- and calcium-deficient medium. The nerve was stimulated at 100 shocks per sec which produces a progressively increasing end-plate response. The upper record was obtained from the surface of a sartorius muscle showing the "smooth" average population response of a few hundred end plates. In the lower part, the response of a single end plate is recorded intracellularly, showing the quantal fluctuations of the response. Stimuli indicated by dots. Note spontaneous potentials on the superimposed "base lines" [from J. del Castillo and B. Katz, J. Physiol. **124**, 574 (1954)].

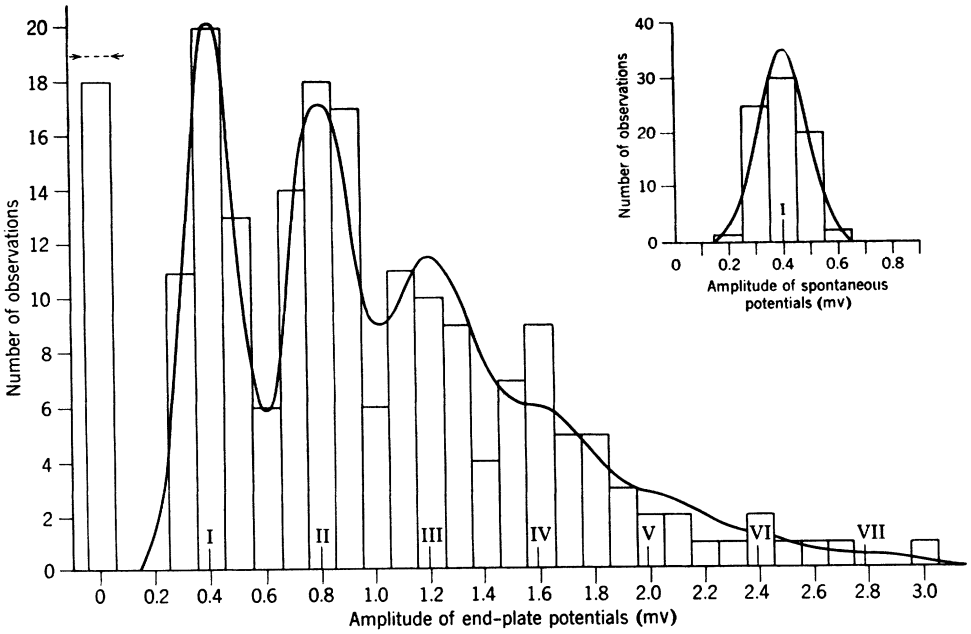

FIG. 5. Histograms of e.p.p. and spontaneous potential amplitudes (inset), from a mammalian end plate blocked by magnesium. Peaks of e.p.p. amplitude distribution occur at 1, 2, 3, and 4 times the mean amplitude of the spontaneous miniature potentials. A Gaussian curve has been fitted to the latter and used to calculate the theoretical distribution of e.p.p. amplitudes (continuous curve). Arrows indicate expected number of failures (zero amplitude) [from I. A. Boyd and A. R. Martin, J. Physiol. **132**, 74 (1956)].

cording equipment, and conversely that a discrete miniature e.p.p., with its standard size and brief time course, must be due to a synchronous package of ACh molecules, may be hundreds or thousands, discharged in the immediate vicinity of our end-plate receptors.

That this packet is the basic coin in which the trans-

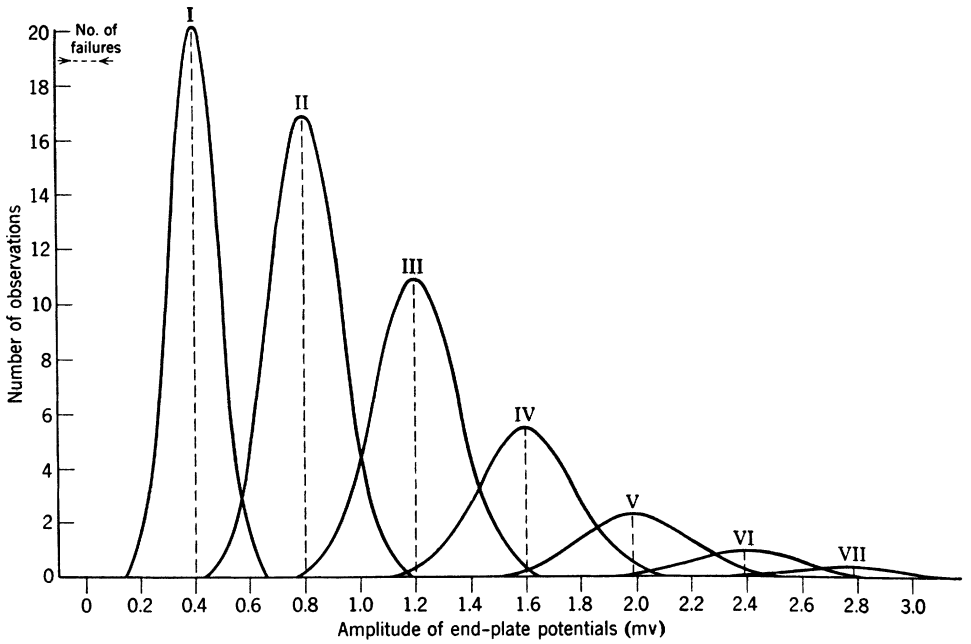

FIG. 6. Method of obtaining the continuous theoretical curve in Fig. 5. A Poisson distribution was calculated, for a mean value m = mean amplitude of e.p.p. responses/mean amplitude of spontaneous potentials. The calculated numbers of each Poisson class have been distributed along Gaussian curves, corresponding to multiples of the spontaneous potentials (see Fig. 5). Algebraic summation of ordinates gives the continuous curve of Fig. 5 [from I. A. Boyd and A. R. Martin, J. Physiol. **132**, 74 (1956)].

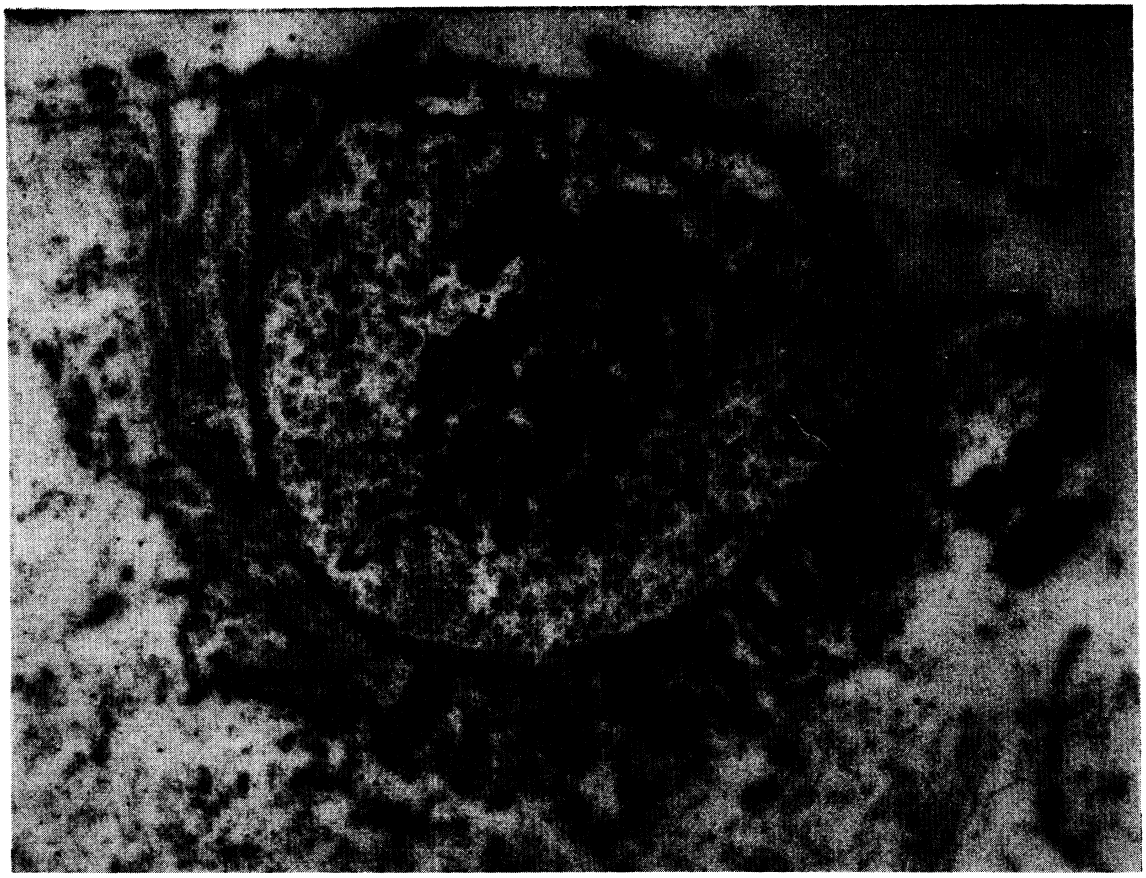

FIG. 7. Electron micrograph of a reptilian neuromuscular junction. Diameter of the motor-nerve ending is approx 2.5 μ; it contains, in addition to mitochondria (large dark particles), many small vesicles of a few hundred A units diameter [from J. D. Robertson, J. Biophys. Biochem. Cytol. **2**, 381 (1956)].

mitter is normally delivered from the nerve endings, has been shown in several kinds of experiments. The release of ACh by an impulse depends upon a number of "co-factors" which can be varied experimentally: among these perhaps the most important are the concentrations of calcium and magnesium in the surrounding fluid. Calcium is an essential adjuvant, magnesium an inhibitor of the release mechanism. By lowering the calcium and raising the magnesium concentrations, the quantity of ACh liberated during an impulse, and the size of the resulting e.p.p., can be progressively reduced towards zero. The point of interest is that, during such an experiment, the e.p.p. at each individual end plate is found to be diminished in discrete steps, which correspond to the dropping out of individual miniature potentials, one by one. With a suitable ratio of Ca/Mg concentrations the liberation can be reduced to a small number of ACh packets;[10] in this condition, the size of the end-plate potential during successive nerve impulses has been found to fluctuate in a characteristic stepwise manner, corresponding to a Poisson-wise distribution of the number of units released in each instance.[11,12] The unit-step is identical with the spontaneously occurring miniature potential. Examples are shown in Figs. 3–5, Fig. 6 showing the method whereby Fig. 5 is obtained; detailed studies of this effect on a variety of vertebrate nerve-muscle junctions have made it certain that transmission is brought about by a summation of many of these quantal units of activity.

A further point of interest is that the size of the unit parcel of ACh which is delivered from the nerve endings, spontaneously or in response to an impulse, is relatively constant at all cell junctions and apparently quite unaffected by the many changes which may be imposed on the system in the course of an experiment. On the other hand, the probability of release of any one parcel in a given time interval—that is, the frequency of the miniature potentials—can be altered by several orders of magnitude, for example, by electrically depolarizing the nerve ending with a steady current, or by changing the chemical composition of the environment. The action of the nerve impulse itself can be described as causing a momentary enormous increase in the frequency of the miniature potentials (by a factor of nearly 10^6, provided a high Ca/Mg ratio exists in the surrounding medium).

The basis of this multimolecular quantum of ACh release is not yet known; an attractive suggestion is that the transmitter substance is stored, within the nerve endings, in minute intracellular corpuscles from which it is discharged at the surface in an all-or-none manner.[6,7] Electron micrographs[13,14] have revealed a mass of fairly densely packed so-called vesicles inside the nerve terminals, and it is conceivable that these are the intracellular bags in which ACh is being stored prior to its release (Fig. 7). It is possible to imagine mechanisms by which a collision between such particles and certain critical spots in the nerve membrane could bring about sudden liberation of the vesicular contents straight into the synaptic cleft. But while it is easy to present speculations which are compatible with the existing evidence, to put them on a firm experimental basis will be a much more difficult task.

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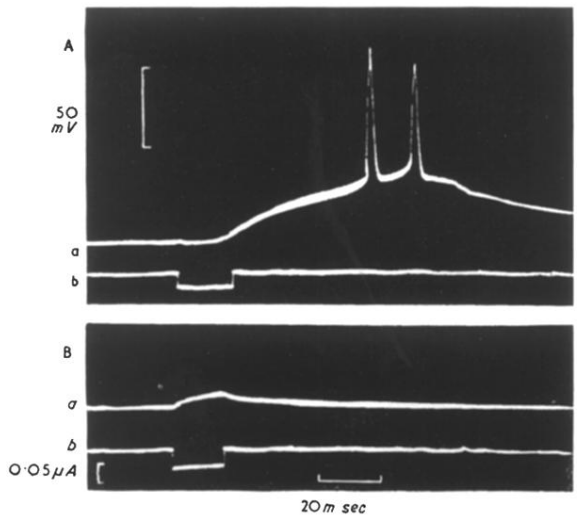

FIG. 1. External and intracellular application of acetylcholine to a motor end plate [from J. del Castillo and B. Katz, J. Physiol. **128**, 157 (1955)]. Intracellular recording of membrane-potential changes from the junctional region of a frog muscle fiber. In *A*, an ACh-filled micropipette was placed on the outside of the end plate, and a quantity of ACh was released by passing a brief outward-directed current pulse through the pipette (registered in *b*). It produced the effect shown in trace *a*: a depolarization developing after a diffusion delay and culminating in two spikes. Between records *A* and *B*, the ACh-pipette entered the muscle fiber. An outward pulse produces now a small, direct potential change, owing to the passage of current through the fiber membrane. (If, for comparison, a KCl-filled micropipette is used, no potential change is produced by the pulse until the pipette has entered the fiber, when the effect is identical with that recorded in *B*, a.)

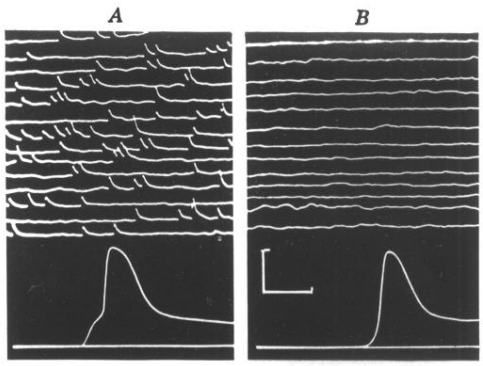

FIG. 2. Spontaneous miniature end-plate potentials. *A:* A recording microelectrode was placed inside a frog muscle fiber at the nerve-muscle junction. *B:* The electrode was placed 2 mm away into the same muscle fiber. The upper portions were recorded with slow speed and high amplification and show the occurrence of spontaneous small potential changes, restricted to the junctional region (calibrations: 3.6 mv and 47 msec). The lower portions show the response to a nerve impulse with fast-speed and low-gain recording (calibrations: 50 mv and 2 msec). The stimulus was applied to the nerve at the beginning of the trace; response *A* (at the end plate) shows the step-like initial end-plate potential which leads up to the propagating wave; response *B* shows only the propagated action potential, delayed by conduction over a distance of 2 mm [from P. Fatt and B. Katz, J. Physiol. **117**, 109 (1952)].

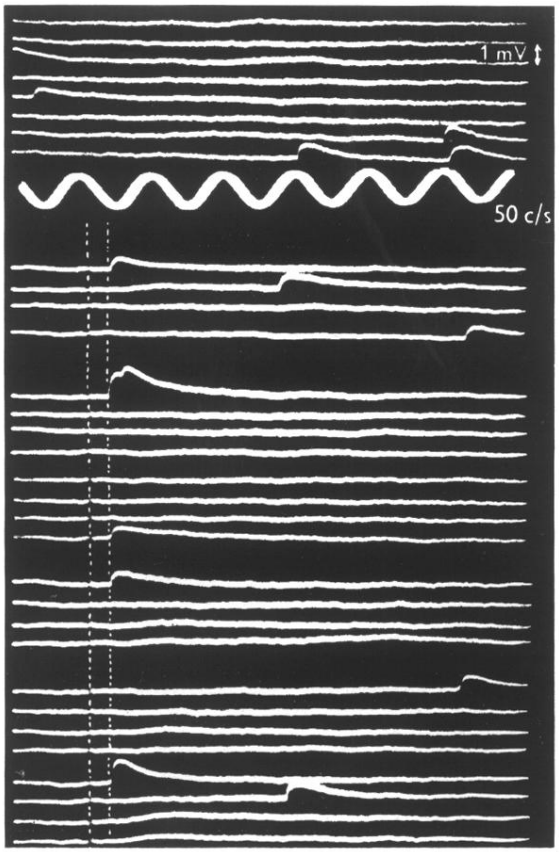

FIG. 3. Quantal units of end-plate response, recorded intracellularly from a muscle fiber in a calcium-deficient and magnesium-rich medium. The top portion shows a few spontaneous potentials. The lower part (below the 50-cps time signal) shows, in addition, the responses to single nerve impulses. Stimulus and response latency are indicated by a pair of dotted lines. There was a high proportion of failures, and only 5 single unit-responses to twenty-four impulses [from J. del Castillo and B. Katz, J. Physiol. **124**, 560 (1954)].

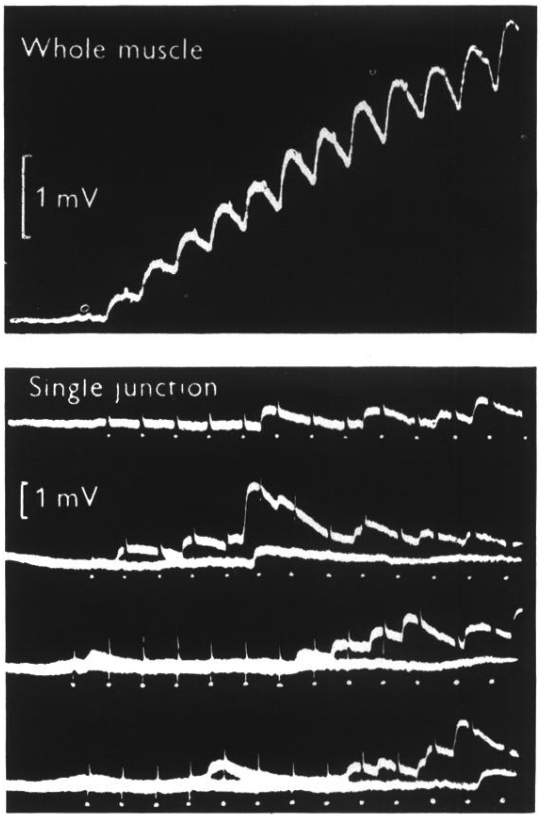

FIG. 4. Statistical properties of the end-plate response. The nerve-muscle preparations were blocked by a high magnesium- and calcium-deficient medium. The nerve was stimulated at 100 shocks per sec which produces a progressively increasing end-plate response. The upper record was obtained from the surface of a sartorius muscle showing the "smooth" average population response of a few hundred end plates. In the lower part, the response of a single end plate is recorded intracellularly, showing the quantal fluctuations of the response. Stimuli indicated by dots. Note spontaneous potentials on the superimposed "base lines" [from J. del Castillo and B. Katz, J. Physiol. **124**, 574 (1954)].

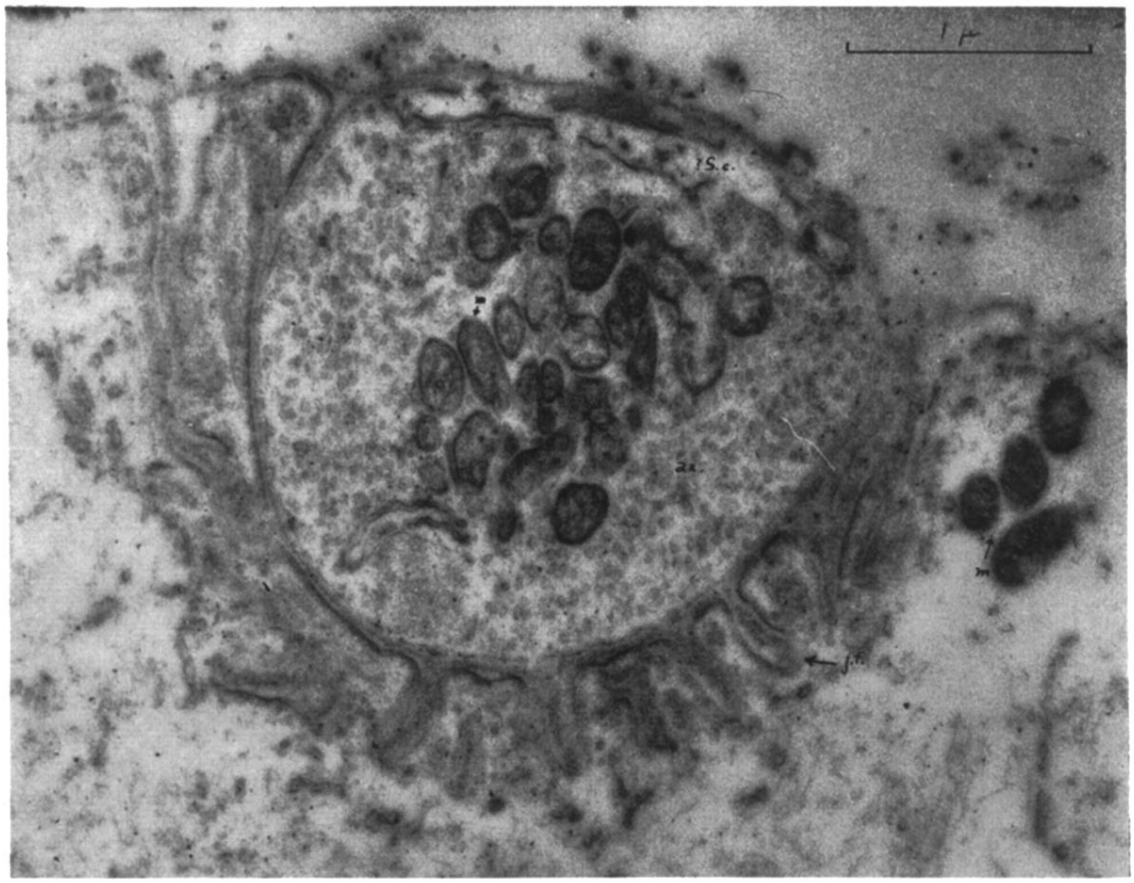

FIG. 7. Electron micrograph of a reptilian neuromuscular junction. Diameter of the motor-nerve ending is approx 2.5 μ; it contains, in addition to mitochondria (large dark particles), many small vesicles of a few hundred A units diameter [from J. D. Robertson, J. Biophys. Biochem. Cytol. **2**, 381 (1956)].