HISTORY OF NEUROSCIENCE

Ian Parker

SYNAPTIC TRANSMISSION

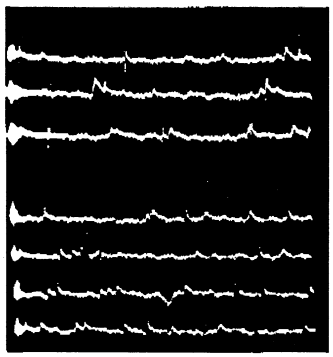
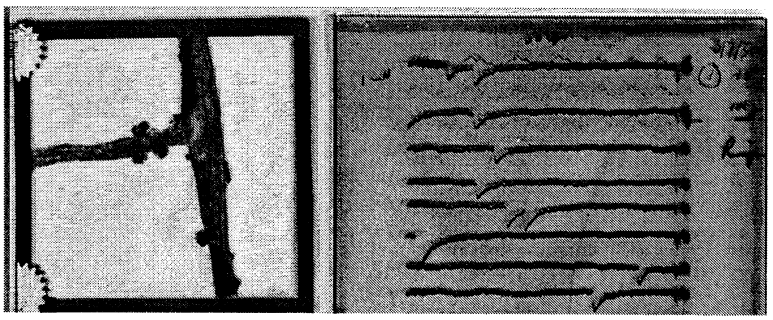


Fig 2. My first 'unwitting' recording of miniature end-plate potentials.
(Katz, 1948)



Functional concept of synapses

Sherrington (1887 - 1906)

" the characteristic features of the reflex arc may be satisfactorily explained by the special properties of the membranes that separate two neurones in close juxtaposition" regions of

In particular this hypothesis proposed to account for one-way conduction in reflex arc, and synaptic delay of 1-2 ms in reflex arc.

Coined term " synapse " (1897)

Greek synapsis — contact, junction

Chemical or Electrical Transmission?

Du Bois Reymond (1877) first suggested that transmission between nerve cells could be electrical or chemical

Long (70 yr!) controversy followed – not resolved until 1950 that transmission at nerve-muscle (n-m) junction and central synapses is chemical.

Major sticking point was rapidity of transmission (1-2 ms)

No such difficulty for autonomic nerves as work much slower (now known to be 2nd messenger mediated). Evidence here for chemical transmission came earlier – but carried little weight for CNS synapses.

Chemical transmission

Early stage of hypothesis

Elliott (1904) suggested sympathetic nerver act by liberating adrenaline onto smooth muscle.

Dixon (1906) parasympathetic nerver may act by liberating muscarine-like substance.

Langley (1892) argued for chemical tranrmission in autonomic ganglia based on blocking action of nicotine

Thus, "stage set and players in place" (Dale) early in century, but electrical theory had strong hold and idear largely forgotten 10 yrs later.

Chemical transmission in heart

Loewi (1921, 26). Stimulated vagus nerve to isolated frog heart – beating slowed. Showed fluid from this heart would slow second heart. "Vagusstoff" – subsequently identified as ACh (acetylcholine).

This experiment at first not well received – tracings were unconvincing; several people could not replicate; concern about introduction of cannula to withdraw fluid.

Kahn (1926) More convincing replication of Loewi's experiment using double-branched cannula.

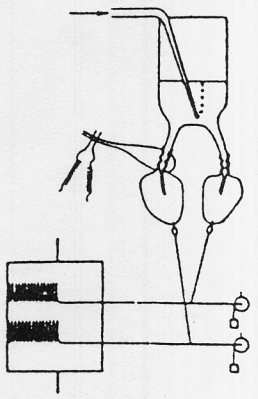


FIG. 3 *a*. — Double branched cannula for studying humoral transmission of vagus stimulation to the frog's heart. Two hearts are suspended on the two branches of the same cannula in such a way that they are bathed by the same fluid. If a vagomimetic substance is liberated on vagus stimulation of the donor heart, the other (receptor) heart should react to it. With this ingenious method any pipetting, as in Loewi's original famous experiment, is avoided. The objection therefore no longer applies that the effect is due to changes in hydraulic pressure.
(From Kahn, *Pflügers Arch. 214*, 485, 1926.)

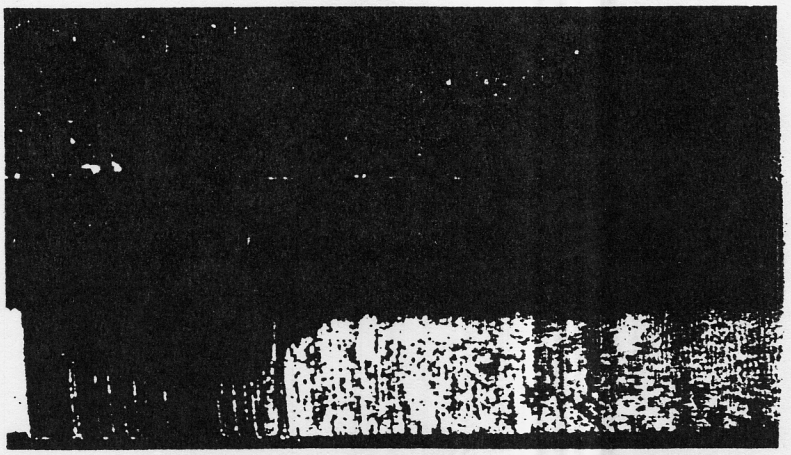


FIG. 3 *b*. Tracings obtained with the method illustrated in figure 3 *a*. Top tracing contractions of the receptor heart; bottom tracing contractions of the donor heart. The signal above the bottom tracing indicates 140 seconds stimulation of the vagus to the donor heart which causes pronounced weakening and slowing of the heart beat. About 70 seconds after the end of this stimulation the contractions of the receptor heart become definitely weaker but not slower.
(From Kahn, *Pflügers Arch. 214*, 488, 1926.)

Extension of chemical transmission to other systems

Cannon, Feldberg, Gaddum, Dale (1931-36)

Obtained strong evidence for chemical transmission in sympathetic ganglia, n-m junction ar well ar in heart.

Eg. Stimulation of motor nerve causes release of ACh, and injection into muscle artery causes contraction (Dale 1936)

But - because transmission at n-m junction very fast compared to autonomic synapses, led Eccles (1936-44) to propose electrical coupling for fast transmission and chemical for prolonged residual depolarization.

Controversy between electrical/chemical transmission provided great stimulus for experiment. Electrical transmission rendered untenable from 1951 by development of intracellular recording.

But - just when chemical transmission established for n-m junction and central synapses Furshpan & Potter (1959) demonstrated electrical synapse in crayfish

Intracellular Recording

Development of microelectrode (Ling & Gerard 1949) revolutionized study of synaptic transmission — allows recording of voltage from single nerve or muscle cell. Earlier extracellular recordings reflect membrane current (not voltage) from (usually) many cells and hard to interpret.

The conversion of Eccles (1951)

Eccles was long-time supporter of electrical transmission. However, his use of intracellular recording gave results impossible to reconcile with this idea — stimulation of inhibitory nerve caused <u>increased</u> polarization of resting potential.

Became enthusiastic convert to chemical transmission

The end-plate potential (e.p.p.).

Fatt & Katz (1951).

By 1951 known that:

nerve impulse → ACh → end-plate potential →

muscle impulse → contraction

Questions were:

What is electric charge passing through end-plate membrane?

How is ACh released by nerve impulse?

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J. Physiol. (1951), 115, 320–370

AN ANALYSIS OF THE END-PLATE POTENTIAL RECORDED WITH AN INTRA-CELLULAR ELECTRODE

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(*Received* 28 *May* 1951)

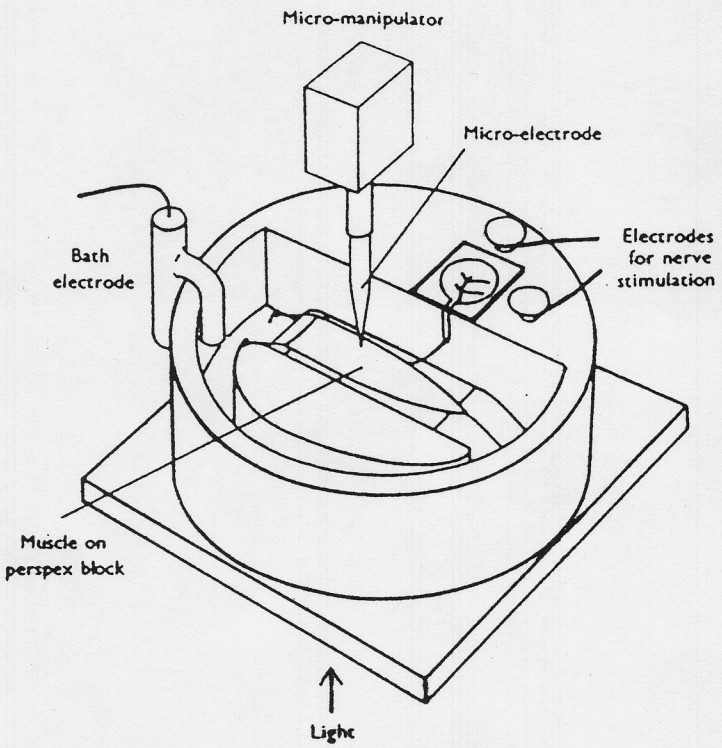

Fig. 1. Nerve-muscle chamber with stimulating and recording electrodes.

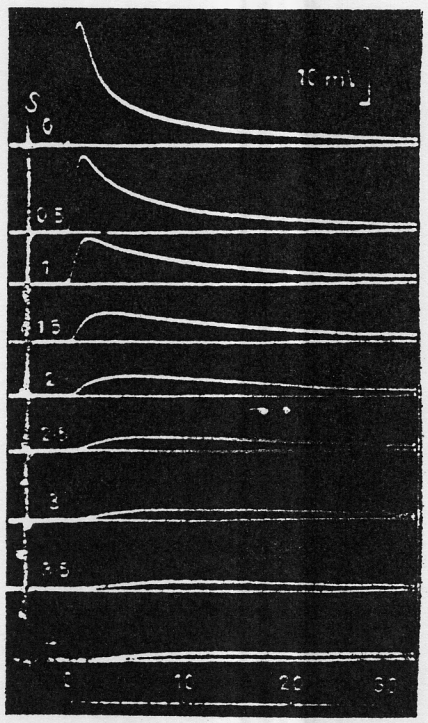

Fig. 5. End-plate potential in a single curarized muscle fibre. The position of the micro-electrode was altered in successive ½ mm. steps. The numbers give the distance from the end-plate focus, in mm. × 0·97. *S*: stimulus artifact. Time in msec.

① Charge passing across end-plate membrane is too great to arise from tiny presynaptic nerve — thus chemical transmission with amplification

② Charge too great to explain by ACh^+ ions crossing muscle membrane. "..... think in terms of some chemical breakdown of a local ion barrier ...."

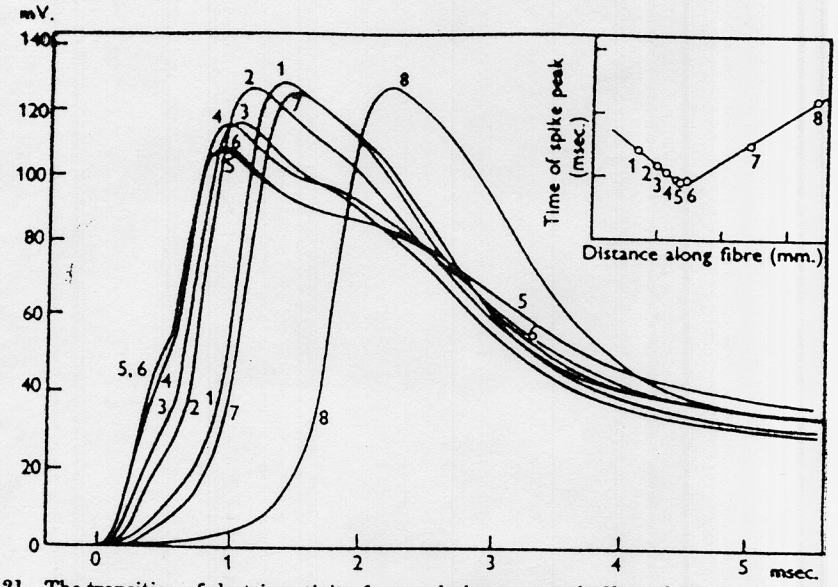

Fig. 21. The transition of electric activity from end-plate to muscle fibre. Calcium concentration, 9 mM. Temp. 17° C. The microelectrode was moved along the fibre, and records were obtained at the following positions (distance from position 1): (1) 0 mm.; (2) 0·3 mm.; (3) 0·45 mm.; (4) 0·6 mm.; (5) 0·65 mm.; (6) 0·75 mm.; (7) 1·75 mm.; (8) 2·75 mm. The resting potential was between 88 and 92 mV. during these records. Note the gradual changes in the shape of the action potential and spike latency. Inset: the time of the spike summit is plotted against distance, showing a propagation velocity of about 1·4 msec. in both directions from positions (5) and (6).

(3) e.p.p. localized to end-plate and spread influenced by cable properties — model for later analysis of integration of synaptic events in neurones

(4) Interaction of e.p.p. and action potential showed e.p.p. caused by increase in conductance ("short-circuit") with reversal of ~ −15 mV. Suggested membrane becomes permeable not only to Na^+ and K^+, but all anions and cations.

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With 9 text-figures
Printed in Great Britain

(14)

ON THE PERMEABILITY OF END-PLATE MEMBRANE DURING THE ACTION OF TRANSMITTER

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(*Received* 25 *April* 1960)

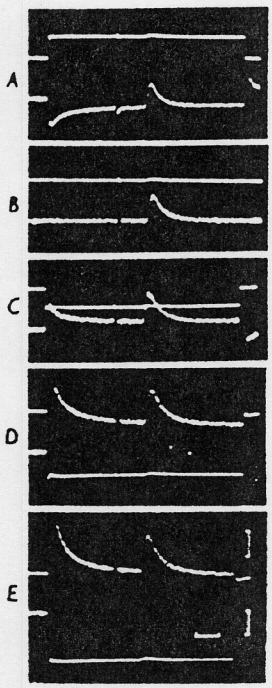

Fig. 1

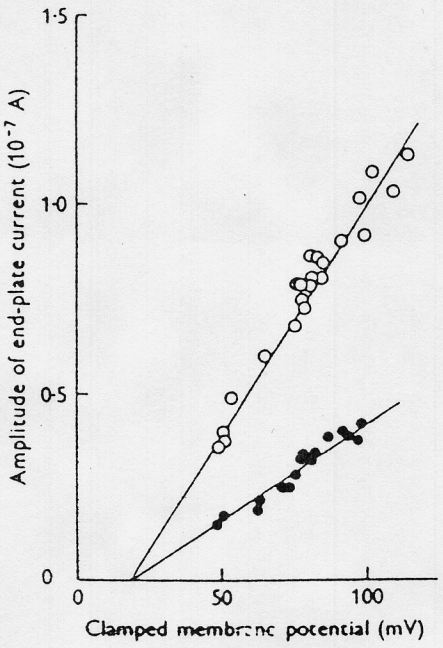

Fig. 2

Fig. 1. End-plate currents and clamped membrane potentials recorded from a curarized end-plate in normal Ringer's solution. Upper traces represent clamped membrane potentials and lower traces feed-back currents containing the end-plate currents. In *A* a square pulse was applied to the feed-back system to depolarize the end-plate membrane. *B*, end-plate current obtained when the membrane was clamped at the resting potential (85 mV). In *C*, *D* and *E* the membrane potential was hyperpolarized to various values. The end-plate currents are superimposed on the current which maintains the membrane potential at various levels. Upper bar, 1×10^{-7}A; lower bar, 10 mV. Time marker, 2 msec. Temperature 20° C.

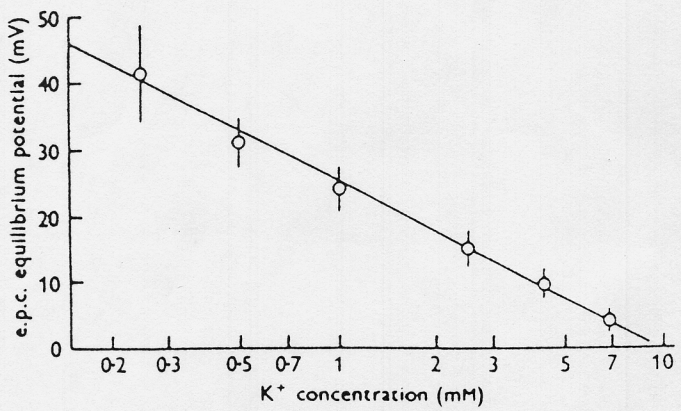

Fig. 7. E.p.c. equilibrium potential plotted against potassium concentration in outside solution on semilogarithmic scale (mean ± S.D.). The line is drawn according to $(1/2{\cdot}29)\{58 \log_{10}(126/K_o) - 1{\cdot}29 \times 50\}$ mV.

Used voltage-clamp to bette estimate reversal potentia of end-plate current.

Found that reversal varied with extracellular $[Na^+]$ and $[k^+]$, but not other ions.

Thus ACh makes musle membrane selectively permeable to Na^+ and K^+ ions.

SPONTANEOUS SUBTHRESHOLD ACTIVITY AT MOTOR NERVE ENDINGS

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(*Received* 5 *December* 1951)

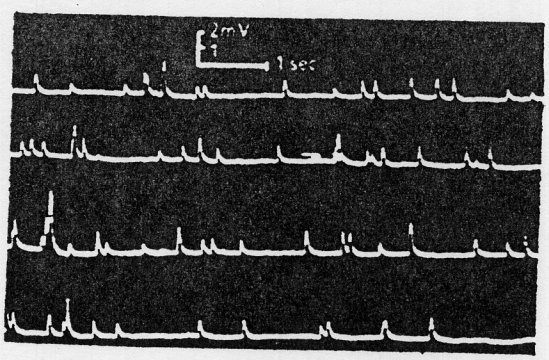

Fig. 2. Example of miniature e.p.p.'s in a muscle treated with 10^{-4} prostigmine bromide.

"Study arose from chance observation ... of spontaneous electrical discharges ...".

Miniature end-plate potentials (m.e.p.ps) seen only at end-plate and abolished by curare — so due to release of ACh by nerve.
Not mimicked by low [ACh], so not due to single molecules of ACh.

Red herring — Following suggestion by Mr. A.L. Hodgin, considered that m.e.p.ps may arise through thermal agitation causing 'noise' fluctuations of voltage in small terminals of nerve : eventually debunked in 1955 (Del Castillo & Katz) as m.e.p.ps. persist when electrical activity abolished by low Na^+ / high K^+ solution.

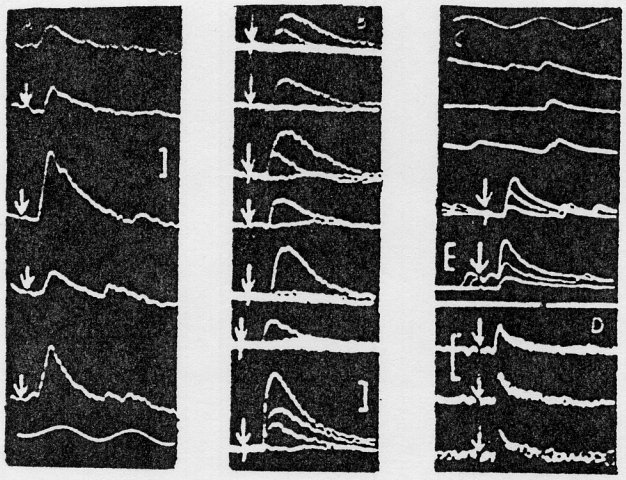

Fig. 9. *Effect of* Ca-*lack*. *A–C*: three different experiments. Muscles soaked in reduced ($\frac{1}{4}$) Ca concentrations; in *B*, prostigmine bromide 10^{-4} was present. Note: the records in *A* and the three top records in *C* were single sweep records. All other records were obtained with multiple 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* (5×10^{-4} D-tubocurarine chloride; three successive records, each with three superimposed sweeps). Volt scale: millivolts. Time: 50 c/s.

"...curious effect observed reducing Ca^{2+} concentration..."

e.p.p. diminished without affecting size of m.e.p.p.
small e.p.p.s evoked with sizes varying in step-like fashion corresponding to multipler of m.e.p.p.s

J. Physiol. (1954) 124, 560–573

QUANTAL COMPONENTS OF THE END-PLATE POTENTIAL

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(*Received* 25 *January* 1954)

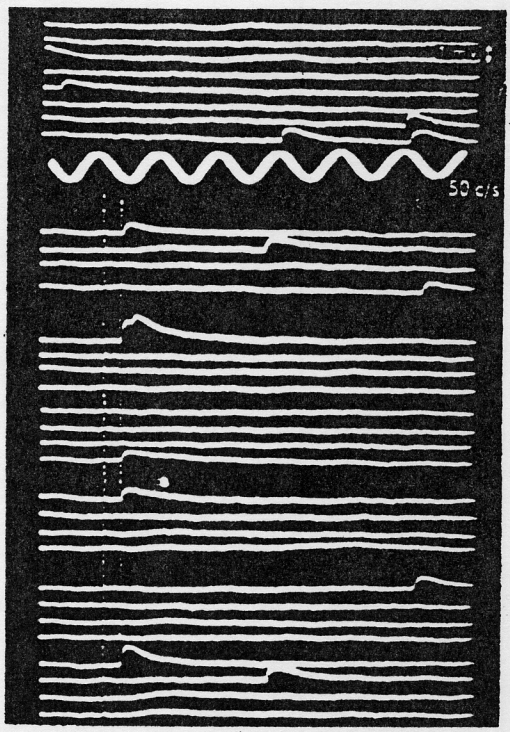
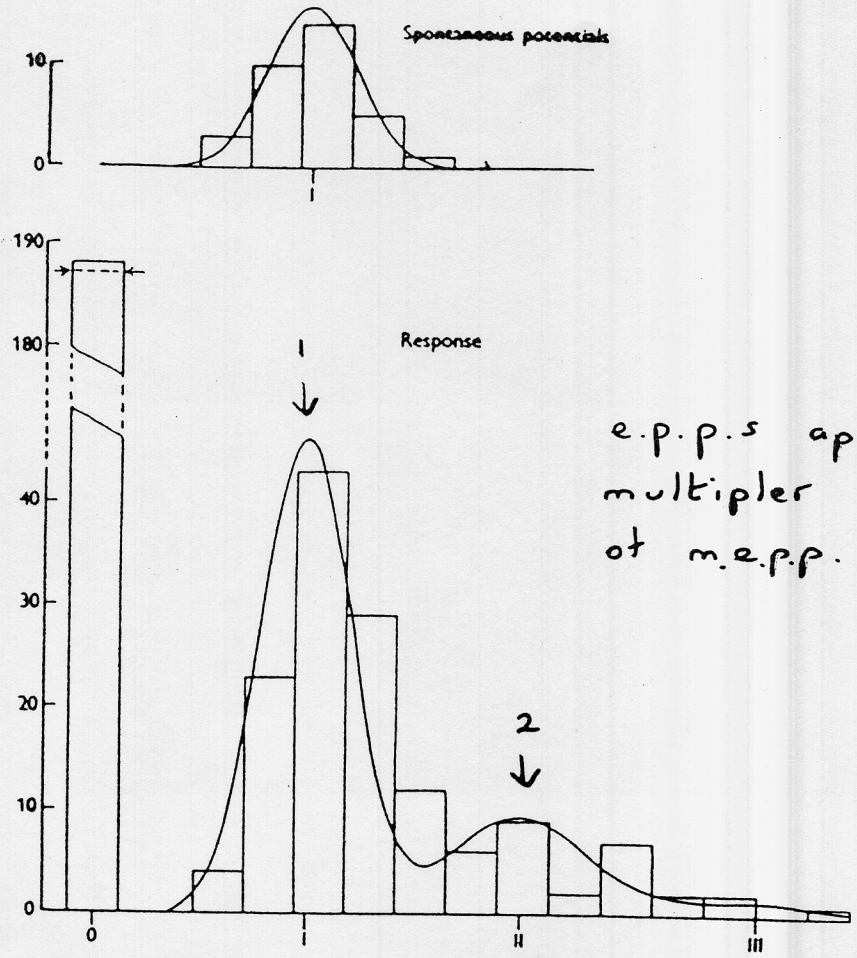


Fig. 2. This muscle was treated with reduced Ca (0·9 mM) and 14 mM-Mg concentration. The top part shows a few spontaneous potentials (traces separated by 1 mV steps). The lower part (below the 50 c/s time signal) shows responses to single nerve impulses. Stimulus artifact and response latency are indicated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

Follow-up to Fatt & Katz (1952)

Propose e.p.p. made up of summation of separate 'parcels' of ACh (~200 in normal conditions) with m.e.p.p. corresponding to least unit or 'quantum'



e.p.p.s appear as multipler of size of m.e.p.p.

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

In this paper no statement of what 'quanta' may represent...

In 1955 synaptic vesicles discovered by electron microscopy (Robertson, de Robertis), suggesting likely morphological correlate of the 'quantum' of ACh.

Lessons.

(1) Importance of development of new, more direct experimental techniques.

may give clear resolution of long-standing controversies

open up unsuspected fields for exploration

(2) Role of 'chance' discoveries : Investigator should do experiments him/herself

(3) Corollary to above – fund people, not short-term specific projects for basic research.