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 18. Also noteworthy is the fact that the cost of labor, as a percent of total expenditures per acre, is lower on farms growing high-yield varieties than on farms producing local varieties (Table 2). Low farm wages, disguised unemployment, and underemployment in Indian agriculture have been explained as a consequence of overutilizing labor, relative to land. See, for example, G. Ranis and J. Fei [*Amer. Econ. Rev.* **51**, 533 (1961)]. The above explanation may be valid when only land and labor are considered as factors of production in developing agricultural economies. However, farmers adopting genetic technologies also use much larger quantities of other inputs—seed, fertilizer, and irrigation. The large absolute and relative increases in the use of these inputs permits large absolute increases in the use of farm labor, without increasing the amount of labor relative to the total package of farm inputs. This implies that the capacity of agriculture in general to absorb labor is enhanced by the adoption of new genetic technologies. Likewise, agriculture's capacity to absorb labor in regions where genetic technologies are applicable is apt to be much larger than in areas where genetic technologies are not applicable.
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Quantal Mechanism of Neural Transmitter Release

B. Katz

I have been asked on more than one occasion to explain the common denominator between the three of us who are sharing this year's award in physiology or medicine. I think the answer is quite simple: The work of all three has a single source, namely the "discoveries relating to chemical transmission of nerve impulses" for which Henry Dale and Otto Loewi received a previous award in 1936. Dale and his colleagues, W. Feldberg, Marthe Vogt, and G. L. Brown, had shown that, in spite of the rapid and unfailing nature of neuromuscular transmission, the motor nerve impulse is not simply

passed on to the muscle fiber by a continuous process of electric excitation, but that there is intervention of a chemical mediator, involving the release from the nerve and the subsequent action on the muscle, of a specific transmitter substance, acetylcholine. This concept is summarized in the following scheme



It was only to be expected that on closer examination this intermediate process would resolve itself into a sequence of reactions made up of a num-

ber of discrete steps, each of which calls for experimental study. What I should like to do in this lecture is to deal briefly with certain advances that have been made during the last 20 years in the investigation of the first stage of the transmission process, namely the mechanism by which arrival of an impulse enables the motor nerve ending to release the transmitter substance. I shall concentrate on studies made by a microelectrophysiological approach and shall refer in particular to the work carried out together with my colleagues Paul Fatt, José del Castillo, and Ricardo Miledi with all of whom I had the privilege to collaborate (1, 2).

Copyright © 1971 by the Nobel Foundation. The author is professor of biophysics at University College, London. This article is the lecture he delivered in Stockholm, Sweden, on 12 December 1970 when he received the Nobel Prize in Physiology or Medicine, a prize he shared with Professor Ulf von Euler and Dr. Julius Axelrod. The article is published here with the permission of the Nobel Foundation and will also be included in the complete volume of *Les Prix Nobel en 1970* as well as in the series Nobel Lectures (in English) published by the Elsevier Publishing Company, Amsterdam and New York. The lectures of Professor von Euler and Dr. Axelrod will be published in subsequent issues.

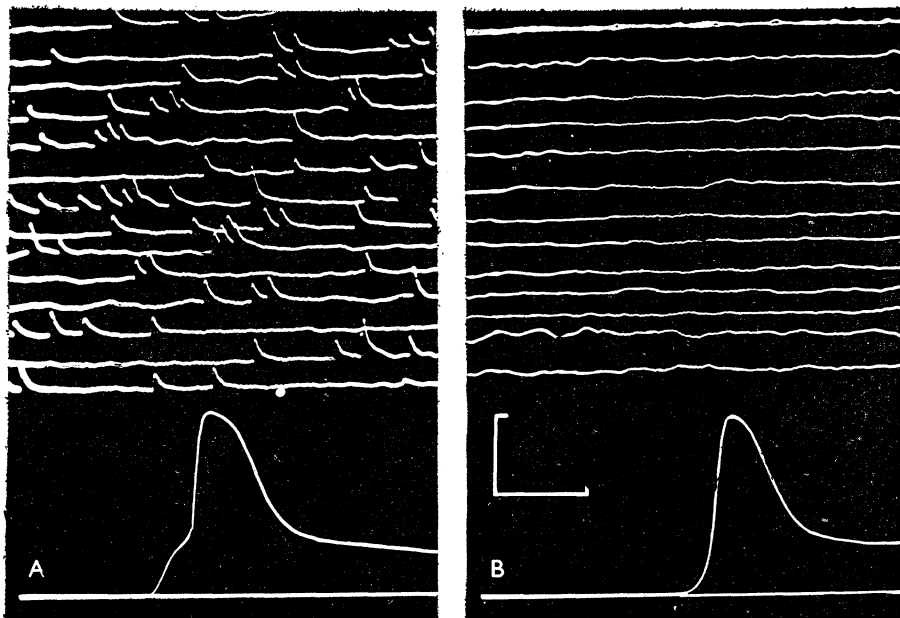


Fig. 1. Spontaneous "miniature end-plate potentials." (A) Intracellular recording at an end plate. (B) Recorded 2 mm away in same muscle fiber. Upper portions were recorded at low speed and high amplification (calibrations 3.6 mv and 46 msec); they show the localized spontaneous activity at the junctional region. Lower records show the electric response to a nerve impulse, taken at high speed and lower gain (calibrations 50 mv and 2 msec). The stimulus was applied to the nerve at the beginning of the trace; response A shows step-like end-plate potential leading to a propagating muscle spike; in B, the spike alone is recorded after additional delay due to conduction along 2 mm of muscle fiber. In all figures, unless otherwise stated, upward deflexion means positive-going potential change at the microelectrode [from (1)].

It had been known for many years (3) that the end-plate region of the muscle fiber, that is, the surface area contacted by the motor nerve, serves as a sensitive chemodetector for a variety of choline esters and especially for acetylcholine. When acetylcholine is applied in small amounts to the outer surface, it opens up ionic channels in the membrane through which ambient cations can pass (4). This allows sufficient current flow to produce a measurable discharge, or local "depolarization" (that is, lowering of the normal membrane potential) of the

muscle fiber. The end-plate surface of the muscle acts, in effect, as a chemoelectric transducer which enables us to register impacts of small quantities of acetylcholine in the form of local potential changes.

Normally, the nerve impulse liberates an amount of acetylcholine that is sufficient to produce a very large local depolarization, often of more than 50 millivolts, the so-called end-plate potential. This rises quickly above the "firing threshold" of the muscle fiber, and thus initiates a new propagating wave of membrane excitation.

Some 20 years ago, using the method of intracellular recording, Paul Fatt and I came across something quite unexpected. In the absence of any form of stimulation, the end-plate region of the muscle fiber is not completely at rest, but displays electric activity in the form of discrete, randomly recurring "miniature" end-plate potentials. Each is only of the order of 0.5 mv in amplitude, but in other respects resembles the much larger end-plate potential evoked by the nerve impulse: it shows the same sharp rise and slow decay, and has the character of a discrete all-or-none phenomenon, although on a much smaller amplitude scale (see Fig. 1).

Numerous experiments have shown that each miniature end-plate potential arises from the synchronous impact of a large multimolecular quantum of acetylcholine spontaneously discharged by the adjacent nerve terminal. Each event is highly localized and involves only a very small portion of the synaptic axon surface; successive discharges form a random sequence in temporal as well as in spatial distribution along the motor nerve ending.

One of the unanswered questions concerns the precise number of acetylcholine molecules which make up each quantal unit of discharge. This is still uncertain; on present estimates one may assume that at least a thousand molecules are contained within an elementary packet, possibly many more. As an upper limit R. Miledi (5) gave a figure of 10^5 molecules; this was based on the minimum quantity of applied acetylcholine which was needed to produce an equivalent effect.

One of the difficulties in answering this question is that neither the chemosensitivity of the end plate nor the resolving power of our recording system is high enough to enable us to detect the effects of individual or of paucimolecular reactions of acetylcholine directly. An indirect approach was chosen quite recently by R. Miledi and me (6). We found that a steady dose of acetylcholine produces not only a measurable depolarization, but also a measurable excess of voltage noise in the end-plate zone. These experiments are still in progress; a preliminary analysis indicates that the elementary "shot effects" which underlie, and statistically build up, the steady acetylcholine potential amount to a fraction of microvolt in amplitude. This elementary voltage change is presumably due to the transient opening of an ionic channel in the muscle membrane, by the

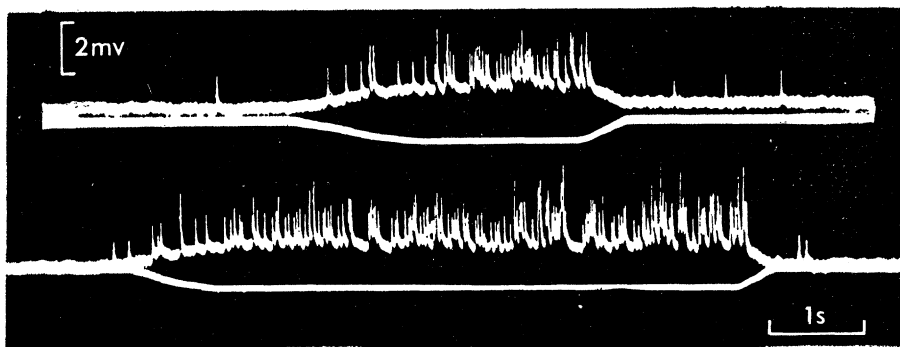


Fig. 2. Electrical control of the frequency of miniature end-plate potentials (11). In each pair of traces, the upper shows miniature potentials, the lower indicates current flowing through the terminal part of the motor axon. The cathode was placed near the junction so as to depolarize the nerve endings. This caused the frequency of the discharge to increase dramatically.

action of one or several acetylcholine molecules. The miniature potential is a thousand times larger and would thus require the opening of one or a few thousand "ionic gates," but this figure still leaves us in doubt about the number of acetylcholine molecules that are discharged in a single quantal unit from the nerve terminal.

The discovery that acetylcholine is spontaneously released from nerve endings in the form of large multimolecular packets acquired further significance as a result of the following findings.

1) The presence of miniature potentials is not peculiar to the neuromuscular junction nor is it confined to cholinergic systems, but has been found to occur at diverse kinds of synapses, in the peripheral and central nervous system, and throughout the animal kingdom. It appears to be a characteristic property of many, maybe of all those neuronal and neuro-effector junctions at which chemical mediation occurs.

2) An important structural correlate has been found by de Robertis and Bennett (7) and by Palade and Palay (8) in the synaptic vesicles which form a distinct population of intraaxonal organelles clustered together near the presynaptic release sites. Recent biochemical studies, especially on the electric organ of *Torpedo* (9) have shown that a major part of the acetylcholine stores of a cholinergic axon is parceled up within these vesicular organelles. Electron microscopic studies on the same tissue, with the freeze-etching technique (10), have revealed many instances in which such vesicles are found to be attached to the presynaptic axon membrane and to have opened into the synaptic cleft.

3) One of the most important findings was that the frequency of the miniature end-plate potentials, that is, the rate of acetylcholine secretion, is controlled by the membrane potential of the axon terminals: depolarization of the presynaptic membrane causes the rate of the discharge to increase in a graded manner without change in the size of the individual "blips" (11) (see Fig. 2). This is, in fact, what occurs after the arrival of an action potential: after a short delay the frequency of miniature end-plate potentials rises by several orders of magnitude for a brief period and then rapidly falls again toward the resting level (12). The result is a very large synchronous end-plate potential which exceeds the "firing threshold" of the muscle fiber.

It has been well established that the

normal end-plate potential is made up of a statistical fusion of quantal components which are identical with the spontaneously occurring units. In effect, the nerve impulse does not start up a new secretory process, but it facilitates or, in statistical terms, raises the probability of events that occur all the time at a low rate, so that—instead of an average of one packet per second—we obtain a few hundred packets of trans-

mitter substance released within a millisecond.

How does the nerve impulse, or more generally how does a depolarization raise the probability of occurrence of this quantal event? The membrane potential change alone is not sufficient. The presence of calcium ions in the external medium is required to make depolarization effective (see Fig. 3). If one progressively reduces the calcium concentration, or adds a "competitive" ion such as magnesium or manganese in increasing amounts, depolarization becomes less and less capable of accelerating the discharge rate of miniature potentials above their resting frequency. Over the last 6 years, experiments by Miledi and myself have led us to conclude that external calcium is the only immediate ionic requirement for depolarization to evoke transmitter release (13).

On our present evidence, the sequence of events may be described as follows: depolarization opens specific "calcium gates" in the terminal axon membrane; this leads to an influx of calcium ions, provided the membrane potential has not been displaced excessively, that is, to or beyond the calcium equilibrium level (14). Having reached the internal surface of the axon membrane, calcium ions then initiate the "quantal release reaction." Up to this point of the argument we are on reasonably firm ground established by electrophysiological experiments. Beyond this point, I must draw on converging observations from ultrastructural and biochemical studies, all of which go to make up a plausible and, I think, very strong hypothesis, namely that the quanta of transmitter molecules are enclosed within synaptic vesicles which undergo frequent transient collisions with the axon membrane, that calcium brings about attachment and local fusion between vesicular and axon membranes, and that this is followed by all-or-none discharge of the vesicular content into the synaptic cleft. To students of neurosecretory processes the postulate of "evacuation" of vesicles or of dense-cored granules from the cell surface is a familiar and well-documented event; in present-day terminology it is usually described as "exocytosis."

If I am asked how all this research, which is developing rapidly along with biochemical, physiological, and cytological microtechniques, is going to influence our understanding of the operation of the central nervous system and

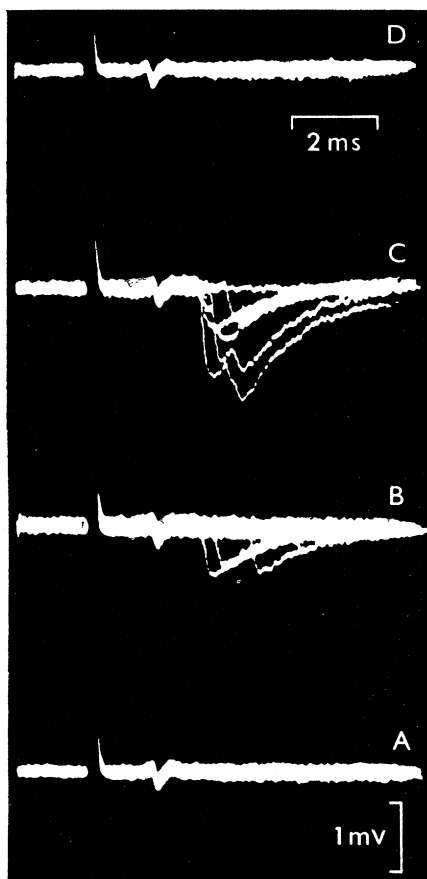


Fig. 3. Exploration of the nerve-muscle junction with a "calcium micropipette" (20). A frog sartorius was immersed in Ca-free solution containing 0.84 mM Mg. A micropipette filled with 0.5M CaCl_2 was used to record focal external potentials from a localized junctional spot. Efflux of Ca was controlled electrophoretically. In the four records, A to D (each obtained by superimposing six traces), Ca efflux was stopped initially by applying sufficient negative voltage to the pipette (in A); the bias was then reduced in two steps (in B and C) allowing increasing amounts of Ca to escape; finally the negative bias was reapplied (in D) to stop Ca efflux again. In A (no Ca), each stimulus is followed at constant interval by a small spike in the nerve terminal, but there is no transmitter release. In B (little Ca), single transmitter quanta were released, after variable "synaptic delay," in three out of the six trials. In C (more Ca), single or multiple transmitter quanta were now released in five out of six trials, while in D (no Ca) transmitter release was stopped again.

of its functional defects, I am on much more tenuous ground. There is some evidence that in certain peripheral neuromuscular syndromes the quantal release efficacy of the motor impulse is impaired (15), resembling somewhat the experimental condition of low Ca-high Mg, while in other forms of myoneuronal disease a "packaging failure," that is, insufficient accumulation of transmitter by individual vesicles has been suggested (16). It would clearly be of interest to pursue this line and to find out whether there is similarly localized involvement in some central nervous lesions.

Finally, there is the more general question, whether the statistical fluctuations and "uncertainties" which are inevitably associated with the quantal nature of transmitter release, play any recognizable role in the organized function of the nervous system. That there are large quantal fluctuations in the response of unitary synapses in the central nervous system, has been shown very clearly by Kuno (17) and others. In many instances, the number of packets released by an impulse impinging on a spinal motoneuron was found to be small, and the predictability of the synaptic response appeared to fol-

low the statistical rules of Poisson's law. In other instances, the fluctuations were much smaller, indicating a greater efficacy of the afferent impulse, so that either a large average number of packets was being discharged from the terminal (18), or a small number was being released with high probability. One would presume that in a "fully trained" neuronal pathway, quantal fluctuations become unimportant because of simultaneous involvement of a large population of synaptic transfer sites. The larger the average number, the smoother and more predictable becomes the synaptic performance. However, large numbers and smooth performance may not be the rule at all times and in all pathways. Experiments on the neuromuscular junction have shown that certain processes of synaptic modification during and after prolonged activity are associated with quantal recruitment, that is, with an increase in number of packages delivered per impulse (19). Similar changes would be expected to occur, and make synaptic performance more predictable, during development and "training," while the opposite trend might underlie some forms of pathological and degenerative impairment.

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NEWS AND COMMENT

Nuclear Reactor Safety: A New Dilemma for the AEC

The Atomic Energy Commission has adopted a curious position lately. While assuring the public that the nuclear reactors it licenses will operate safely, a number of AEC officials have been discreetly appealing for more money—preferably much more money—to support research on the safety of conventional, water-cooled nuclear reactors.

For the most part, the money has not been forthcoming. But the appeals themselves have inflamed suspicions among the AEC's numerous critics that the atomic power plants that are blossoming across the nation's landscape may not be as secure from mishap as licensing implies. And thus the AEC finds itself impaled on a new

dilemma that may serve to weaken its already shaky public credibility: If reactors are as safe as they are advertised to be, how can a large new safety budget be justified? Or, if new safety research is as urgently required as the AEC indicates, should the construction of atomic power plants (21 are operating, more than 50 are being built) be proceeding as rapidly as it is?

At a time when the AEC's \$3 billion program for subsidizing the development of water-cooled reactors is nearly at an end, and when new plants are being licensed in ever-increasing numbers, the commission's appeals to Congress and the Office of Management and Budget for safety funds seem more than a little awk-

ward. Indeed, the AEC's rationale for funds above and beyond its fiscal 1971 safety budget of \$36 million rests partly on the premise that significant "uncertainties" in the performance of reactors remain, and that "urgent" work is yet to be done to resolve these uncertainties. For example, Milton Shaw, the AEC's director of reactor development and technology, confides that "At the drop of a hat I can spell out 15 areas where we could do more research in reactor safety. Drop two hats and I'll spell out 30 areas. There's virtually no limit on the work we can do." At the same time, however, commission officials are at pains to deny any untoward implications in such statements. In this context, George M. Kavanagh, the assistant general manager for reactors, told the Joint Committee on Atomic Energy last March that "This does not mean our reactors are unsafe. It means we should be spending more to assure that they are safe. . . ."

One way out of this paradox is to concede the point, raised by those close to reactor development, that safety research is not a finite task. As reactors age and their designs evolve, so the job