Proc. Nat. Acad. Sci. USA
Vol. 70, No. 3, pp. 727–731, March 1973

An Attempt at an Integral Interpretation of Nerve Excitability

(basic excitation unit/proteins and bioelectricity/acetylcholine receptor/threshold/ synaptic transmission)

EBERHARD NEUMANN*, DAVID NACHMANSOHN†, AND AHARON KATCHALSKY‡

* Max-Planck-Institute of Biophysical Chemistry, Goettingen, Germany; † Departments of Neurology and Biochemistry, Columbia University, New York, N.Y. 10032; and ‡ Polymer Department, The Weizmann Institute of Science, Israel

Contributed by David Nachmansohn, January 4, 1973

ABSTRACT **A qualitatively consistent integral interpretation of biochemical, electrophysiological, and biophysical data on nerve activity is given in terms of a basic excitation unit. This operational term models a dynamically coupled assembly of membrane components accounting for graded and all-or-none responses upon stimulation. The analysis contains a series of suggestions linking controversial interpretations and is aimed at stimulation of experimental studies providing the basis for a quantitative integral theory of nerve excitation.**

The electrical excitability of nerve cells continues to be an exciting challenge to science. Although an overwhelming amount of information has accumulated on various aspects of nerve activity, the molecular mechanism for generation and propagation of nerve impulses is not yet understood. The numerous experimental observations suggest that nerve activity must be based on a complex chain of various elementary processes. It seems obvious that any *quantitative* theory of bioelectricity should be preceded by an *integration* of the basic electrophysiological, biochemical, and biophysical data into a (at least qualitatively) consistent picture.

Such an integral approach was initiated by the late Aharon Katchalsky, and the coauthors present in this report qualitative correlations between some of the basic facts established in different fields concerned with nerve activity.

The approximate and selective form of many quantitative descriptions of certain aspects of nerve excitation will become apparent when we inspect some of the well-known chemical and electrochemical properties of excitable membranes. Although the observed electric potentials and potential changes reflect membrane processes, electric excitability is not solely a membrane phenomenon, but is intrinsically coupled to the metabolic activity of the nerve cell. In general, a living organism is characterized by a high degree of coupling between energy and material flows. Similarly the nerve cell is a nonequilibrium system and, furthermore, the specific function of its membrane requires a nonequilibrium distribution of the cell components. The anisotropic ion distribution across the nerve membrane is the result of a balance of active and passive transport. Thermodynamically, active transport can be viewed as a movement against a chemical or electrochemical potential gradient, mediated by a metabolic reaction. According to the Curie principle, such a chemo-diffusional coupling can only occur in an anisotropic space (1). Anisotropy is apparently a characteristic property of biomembranes. Indeed, the physicochemical behavior of cellular membranes, including axonal membranes, may be described in terms of a layer structure. It was found that the internal layer of the axonal membrane consists of proteins required for excitability (2). Proteolytic action on this layer causes irreversible loss of excitability. The intramembrane structure probably comprises several ionic constituents; fixed charges (some of them may facilitate permselective ion diffusion), mono- and divalent ions, especially Ca^{2+}, and in particular the acidic and basic side chains of the proteins directly involved in the processing of acetylcholine. In general, the different regions of the membrane will have different permeability characteristics, and the steady flow of ions may cause either ion accumulation or depletion within the membrane. This leads to nonlinear dependencies between certain physical parameters, e.g., between current and potential. As a result of this nonlinearity, rectification of current is observed. Corresponding to current rectification there is, for instance, not a straightforward dependence of the membrane potential, $\Delta\psi$, on the logarithm of ion concentrations, i.e., the Nernst equation is not obeyed (1, 2). (This is not surprising because the Nernst equation, adequate for the limiting case of ideal electrochemical equilibrium across an ideal permselective membrane, may only approximately describe nonequilibrium states of real, biological membranes.) Nonequilibrium thermodynamics provides a formal relationship for the steady state of zero net flow ($I_m = 0$) of ions across the membrane:

$$(\Delta\psi)_{I_m=0} = \Delta\psi_D + \frac{RT}{F}\sum_j \frac{t_j}{z_j} \ln \frac{a_j^{(o)}}{a_j^{(i)}}. \qquad [1]$$

In Eq. 1, $\Delta\psi_D$ are the Donnan contributions due to fixed surface charges, R is the gas constant, F is the Faraday constant, T is the absolute temperature, a_j is the thermodynamic activity (outside and inside the nerve membrane, respectively) of the ion j of valency z_j, and the transference number t_j represents the fraction of current carried by the j ions in the membrane (3); t_j and $\Delta\psi_D$ depend on the membrane structure and are therefore not *a priori* known. With $\Delta\psi_D = 0$ and $t_j \simeq 1$, Eq. 1 simplifies to the Nernst equation for j:

$$(\Delta\psi)_{I_m=0} = \frac{RT}{z_jF} \ln \frac{a_j^{(o)}}{a_j^{(i)}}. \qquad [2]$$

A well-known expression of nerve activity is the action potential, resulting from ion flows across the nerve membrane. There is first a rapid increase of Na^+ ion permeability (referred to as "Na activation") leading to an influx of Na^+

Abbreviations: BEU, basic excitation unit; S, storage protein; R, AcCh receptor; E, AcCh esterase; AcCh, acetylcholine.

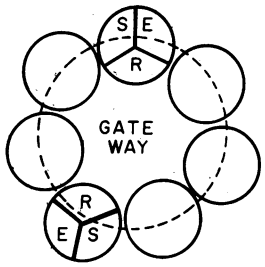

FIG. 1. Scheme of the basic excitation unit (BEU); *S*, storage protein; *R*, receptor protein; *E*, acetylcholinesterase.

ions into the axon interior, followed by a rapid decrease of Na^+ ion permeability ("Na inactivation") and an outflux of K^+ ions (5). The ion fluxes are driven by the concentration gradients maintained by metabolic energy. The fundamental question arises of how this source of potential energy suddenly becomes effective and what is the molecular mechanism underlying the permeability changes.

It is now widely recognized that the present electro-diffusion models are inadequate to describe nerve excitability (4). Since details of the nerve membrane anisotropy are, however, not known, any quantitative nonequilibrium analysis of the ion flows across the excitable membrane faces great difficulties for the time being. The strong heat production and absorption coinciding with the action potential suggests that chemical reactions must be associated with the changes in ion permeability permitting the ion fluxes (6). Recent theories describe the permeability changes during nerve activity in terms of cooperative structural changes in macromolecular membrane components involving ion exchange [e.g., Tasaki (2), Adam (7)]. There is, however, biochemical evidence that nerve stimulation may not directly induce ion exchange and changes of the membrane structure. The basic question remains: what are the preceding events?

I. Chemical theory of excitability

Biological membranes are the site of some of the most vital cell functions and the frame of a large number of chemical reactions. It is now widely accepted that many biomembranes comprising generally some 30–40 different proteins (including enzymes), a great variety of phospholipids, oligosaccharides, etc., are not only passive barriers, but extremely active and dynamic chemical structures.

During the last decades a chemical theory of nerve excitation has been developed by Nachmansohn (8). This approach is the first attempt to correlate biochemical and electrophysiological data. According to the chemical theory, nerve activity is intrinsically linked to the processing of acetylcholine (AcCh) *within* the excitable membrane. On proper stimulation, AcCh is released from a storage site, presumably a protein, and acts on a specific AcCh-receptor protein, thereby inducing a conformational change. This change may release, e.g., by allosteric action, Ca^{2+} ions bound to the receptor. The Ca^{2+} ions in turn may effect conformational changes of membrane constituents (such as proteins, phospholipids, or oligosaccharides). In this way local changes of permeability may be produced that permit ion fluxes across the membranes. At the same time (or subsequently), AcCh is hydrolyzed by AcCh-esterase, permitting the return of the receptor to its resting condition, thereby reestablishing the ion barrier. According to this concept, the release of AcCh is the signal that induces a series of chemical reactions responsible for the increased permeability of the membrane (8). It has been estimated that, per molecule of AcCh released, many thousands of ions, possibly as many as 20,000–40,000, flow in each direction across the membrane.

Among the basic facts underlying the chemical theory is the well-established presence of large amounts of the two specific proteins, AcCh-receptor and AcCh-esterase in all excitable membranes throughout the animal kingdom. For example, in the excitable membrane of a single cell of the electric organ of *Electrophorus*, about 10^{11} molecules of AcCh-esterase are present; the number of AcCh-receptor molecules is in the same order of magnitude. If we assume that the excitable membrane of the electroplax covers an area of about $10 \times 1 \times 0.2$ cm^2—the factor 10 is an estimate, and it refers to surface increase due to extensive invagination—the average distance between AcCh-esterase–receptor assemblies is about 450 Å. One of the particularly striking and most important observations is the fact that the blocking of the AcCh-receptor by antimetabolites of AcCh (such as local anesthetics) abolishes electrical activity in all excitable membranes. In isolated fragments of conducting parts of excitable membranes, forming microsacs, chemical stimulation of the AcCh-receptor protein has similar effects on the ionic parameters (fluxes, conductances, permeabilities), as has been previously found by electrical stimulation of intact axons (9).

II. The integral model of nerve excitability

The present analysis is an extension of the chemical theory and concentrates on the sequence of events leading finally to the induction of permeability changes. The interpretation especially covers (*i*) the observation of a threshold for the generation of an action potential, (*ii*) the problem of all-or-none versus graded responses to stimulation, and (*iii*) the stimulus effect itself.

As mentioned before, the resting nerve cell is in a steady state reflecting a balance between different flows. Modern theories in molecular biology, however, regard living organisms as quasi-stationary with oscillations around a steady average, rather than as steady-state systems (1). For our present purpose it appears adequate to assume that the membrane of the nerve cell is in a stationary state with continuous local activity on a subthreshold level. This view may be clarified by the following considerations.

In some cells the threshold of potential change required for triggering an action potential is known to be about 20–30

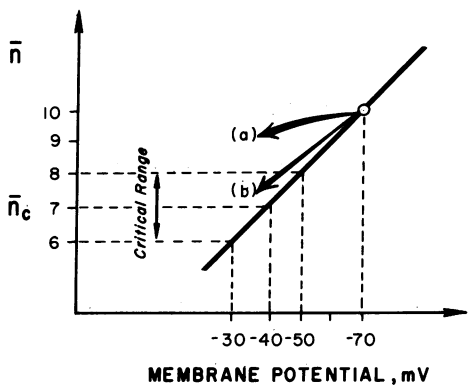

FIG. 2. The function $\bar{n} = f(\Delta\psi)$; see text. (*a*) fast potential change: $\bar{n} \gg \bar{n}_0$, (*b*) slow potential change: $\bar{n} \simeq \bar{n}_c$.

mV. This voltage change corresponds to an energy input per charge of about 1 kT unit (k = Boltzmann constant; T = absolute temperature) at body temperature. It is thus possible that thermal fluctuations cause occasional release of $AcCh^+$ ions. It is intriguing to speculate whether the miniature potentials observed at certain postjunctional parts of excitable membranes are reflections of occasional AcCh release.

1. Basic Excitation Unit (BEU). Our integral model of excitability comprises the assumption of a basic excitation unit. Such a unit is suggested to consist of a gateway that is surrounded by several basic protein assemblies (SRE). The basic protein assembly is most probably an interlocked complex of AcCh-receptor (R), AcCh-esterase (E), and the storage protein (S) for AcCh. The gateway is an operational term describing a dynamically coupled membrane state, a region with probably fixed, mainly negatively-charged groups, and mono- and divalent cations. This gateway is the suggested permeation site for ion movements during excitation. The basic excitation unit is modelled in Fig. 1. The BEUs are assumed to be distributed over the whole excitable membrane. The density of these units may vary according to specific functional requirements. If we assume that one BEU in the electroplax comprises, say, about ten SREs, the average distance between the gateways is about 1400 Å. Thus, the part of the membrane involved in permeability changes during electrical activity is rather small. The low density of permeation sites may well be the reason for the exceedingly small capacitance changes observed during excitation (10).

The introduction of a basic excitation unit becomes useful for the modelling of cooperativity on a subcellular level. We assume that the action potential is based on cooperativity between the SRE subunits of one BEU. In order to initiate an action potential a certain critical number (the cooperative number) of receptors has to be activated within a certain time interval. During this time (latency phase) at least, say six or eight out of about ten basic protein assemblies of an excitation unit must start to process AcCh through the SRE units.

2. Macromolecular Conformation and Ca^{2+} Ions. Before proceeding to more specific points, some physicochemical aspects of macromolecular conformational changes in connection with Ca^{2+} ions are recalled. Structural changes in proteins and macromolecular organizations such as membranes are very often strongly cooperative in nature. One of the consequences of cooperativity is the possibility of far-reaching conformational changes by only small changes in the environmental conditions. Furthermore, cooperative conformation changes induced, e.g., by binding of ligands at one site, may change the reactivity of other, even far remote sites of a macromolecular system (allosteric effect). Ca^{2+} ions are particularly effective in inducing large configurational changes as, for instance, contractions, especially in systems that contain regions of a relatively high negative surface charge. In these polyelectrolyte-like ranges, the osmotic coefficient for Ca^{2+} is in the order of 0.01, i.e., about 99% of Ca^{2+} counterions are "bound" (11). The generally high binding capacity is the reason why Ca^{2+} ions are believed to play a prominent role in maintaining structural and functional integrity of protein and lipoprotein organization. As discussed by Tasaki (2), divalent ions, such as Ca^{2+}, are absolutely necessary for electric excitability in axonal membranes.

3. Threshold. In many cells the steady state of the resting

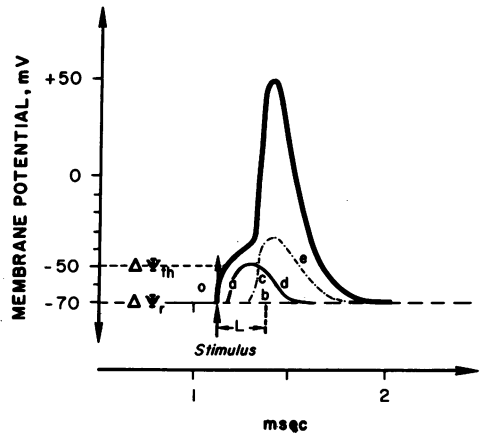

FIG. 3. Schematic representation of an action potential. (*o*) Potential change due to superposition of the above-threshold stimulus potential. (*a*) and (*d*), time course of AcCh release and hydrolysis, respectively; (*b*) and (*c*), time course of Ca^{2+} release from receptor and of gateway opening; (*e*), time course of Ca^{2+} reuptake and conformational relaxation leading to closure of the gateway. *L*, latency; $\Delta\psi_r$, resting potential; $\Delta\psi_{th}$, threshold potential.

excitable membrane is physically characterized by a membrane potential of about −60 to −70 mV. With an average membrane thickness of about 100 Å, this potential difference is assumed to correspond to an average field intensity of about 60–70 kV/cm across the membrane; the field vector is directed from the inside to the outside of the cell. In order to trigger a nerve impulse, a stimulus has to be applied that reduces the membrane potential below a threshold value within a certain time interval. Such a transient reduction may be induced by an electric impulse. In this way a nerve impulse, in its rising phase, is able to trigger action potentials in adjacent regions of the membrane. (This is very plausible since electric fields are known to represent long-range forces.)

An action potential will, however, only be generated by a proper stimulus when the resting potential is held above a certain threshold range of about −40 to −50 mV. In the simplest case, the average number of AcCh, $\bar{n}$, that is bound to the storage site could be linearly dependent on the membrane potential $\Delta\psi$, as depicted in Fig. 2. The function $\bar{n} = f(\Delta\psi)$ reflects steady states of balance between occasional release of AcCh and supply of AcCh to the storage sites. The range $\bar{n}_c \pm \Delta n$ is the threshold range, where Δn may be about ± 1 or 2. For $\bar{n} < \bar{n}_c$ it is no longer possible to activate the cooperative number, $\bar{n}_c$, of receptors in one basic excitation unit.

This interpretation requires the assumption that the binding of AcCh is dependent on the conformation of the storage protein; the binding-conformation being favored at higher membrane fields. If the membrane field is reduced slowly, random discharge of storage sites occurs. The probability that the cooperative number of storage sites release AcCh within a certain time interval is rather low. Thus, corresponding to experience, slow reduction of the membrane field is very unlikely to induce an action potential. Similarly, the decline in excitability known as accommodation is suggested to result from temporary AcCh "exhaustion" of some storage sites.

The liberation of AcCh from the intact storage protein is probably due to a conformational change induced by the

stimulus. This suggestion is based on the following observations. Electric impulses in the order of 20 kV/cm (corresponding to 20 mV/100 Å) are capable of inducing conformation changes in macromolecular organizations of relatively high surface charge. In such systems the electric field displaces the screening counter-ion atmosphere; thereby the repulsion between the charged components is increased and separation of ionic groups may occur (12, 13).

4. Stimulus. As already mentioned, the generation of an action potential requires the reduction of the intrinsic membrane potential $\Delta\psi_r$ to a threshold value $\Delta\psi_{th}$. This potential decrease, $\Delta(\Delta\psi) = \Delta\psi_r - \Delta\psi_{th}$, has to occur in the form of an impulse, $\int\Delta(\Delta\psi)dt$, in which a certain relationship between the membrane potential $\Delta\psi$ and time, t, must be fulfilled. The condition for the initiation of an action potential may then be written:

$$\Delta\Psi_r - \frac{1}{\Delta t}\int\Delta(\Delta\psi)dt \leqslant \Delta\Psi_{th} \qquad [3]$$

where Δt is the pulse duration.

The potential change $\Delta(\Delta\psi)$ is equivalent to a change in the intrinsic membrane field E, defined by $\Delta E = -\Delta(\Delta\psi)/d$, where d is the membrane thickness. From Ohm's law we have $d\cdot\Delta E = -R_m\cdot I$, where R_m is the membrane resistance and I is the current intensity (that could cause a change in E). If we include Eq. **1** or **2**, we obtain:

$$\int\Delta(\Delta\psi)dt = -d\int\Delta E dt = \int R_m\cdot I\cdot dt = \frac{RT}{z_jF}\int\Delta\ln\frac{a_j^{(o)}}{a_j^{(i)}}dt \qquad [4]$$

In Eq. **4**, we see how the intrinsic membrane potential may be changed: by a field (voltage or current) pulse or by an "ion pulse" involving those ions that determine the membrane potential. Such an "ion pulse" may be produced if the external K^+ ion concentration is sufficiently increased within a certain time interval.

5. Latency. The time interval between the onset of the above-threshold stimulation and the appearance of the action potential is called the latency phase (see Fig. 3). The length of the latency is determined by the stimulus intensity. Within the latency time interval the cooperative number of AcCh is released; the release process covering the critical number is shorter the more intensive the stimulus is.

6. Key Processes. According to our model, nerve activity comprises the following key processes:

(*a*) *Release of AcCh.* By a proper stimulus AcCh is released from the storage site, S.

$$(AcCh)S_1 = AcCh + S_2$$

On an elementary scale, this reaction also comprises the field-dependent conformational equilibrium between S_1 and S_2. After release, AcCh is translocated to the receptor.

(*b*) *Binding of AcCh by the receptor.* The receptor is believed to bind Ca^{2+} ions in the AcCh-free state; a portion of Ca^{2+} may be kept in polyelectrolyte patches. If the bound Ca^{2+} ions locally exceed the negative fixed charges, they can provide the "fixed" positive charges accounting for current rectification in the resting steady state (7). The fixed negative charges may facilitate cation movement and prevent anion passage. The binding of AcCh is suggested to induce a conformational change from the Ca^{2+}-binding form to the AcCh-binding configuration

$$AcCh + R\ (Ca^{2+}) = R(AcCh) + Ca^{2+}$$

If the conformational change of the receptor increases the average distance between the negatively charged groups, Ca^{2+} ions will also be released from polyelectrolyte-like regions.

(*c*) *Gateway processes.* The receptor is probably a part of the gateway. The Ca^{2+} ions released may be partially replaced by other cations, e.g., Na^+ ions. Cation exchange may induce local conformational changes in gateway components and lead to local permeability changes that may be responsible for subthreshold responses. If, however, the cooperative number of receptors is activated, ion exchange may induce cooperative structural changes in all gateway components. In this way the permeability of the gateway can be drastically altered: ions and also nonelectrolytes may pass through the membrane.

(*d*) *Hydrolysis of AcCh.* The receptor is assumed to translocate the bound AcCh to the AcCh-esterase where the ester is hydrolyzed.

(*e*) *Conformational relaxations.* After the translocation of AcCh, the receptor is able to relax to its resting conformation permitting reuptake of Ca^{2+} ions; this leads to the closure of the gateway and to the reestablishment of the resting steady state.

The time course of the action potential is sketched in Fig. 3 in terms of the underlying chemical processes after stimulation.

7. Synaptic Transmission. Current flow from the nerve terminal towards the postjunctional side, previously questioned, has now been demonstrated (14–16). This observation is of particular interest in the view of the earlier finding of K^+ efflux at nerve endings (17). In 1935, Eccles suggested K^+ ions to be the transmitters crossing the synaptic cleft (18).

It is instructive to estimate the transient increase of K^+ ion concentration within the cleft volume per impulse arriving at the terminal.

The innervated, excitable part of electroplax carries at least about 20,000 synapses. Since about 0.5% of the membrane surface of about 2 cm^2 is estimated to be synaptic (8), the area covered, on the average, per single synapse is about 5×10^{-7} cm^2. If we assume an average width of the cleft of about 250 Å, the volume of a synaptic cleft is about 1.3×10^{-12} cm^3. We assume that per nerve impulse at least about 10^3 AcCh molecules are activated within the presynaptic membrane (8). Each AcCh may displace at least about 20,000 K^+ ions into the cleft. The transient concentration increase of K^+ ions is estimated to be $(10^3\times2\times10^4\times10^3)/(6\times10^{23}\times1.3\times10^{-12})\simeq0.026$ M. With $z_j = +1$ and $T = 310°K$, we obtain from Eq. [2] that an increase of the outside K^+ concentration from about 0.005 M in the resting state to $a_{K^+}^{(o)}\simeq c_{K^+}^{(o)}\simeq0.026$ M corresponds to a potential change $\Delta(\Delta\psi)\simeq-44$ mV. A potential change of this magnitude may well induce electric activity in the postsynaptic membrane. The figures given for the junctions of the electroplax are necessarily rough approximations. They are, however, useful for the present purpose. [It was estimated by Frankenhaeuser and Hodgkin (19) that even 20 msec after an impulse

the K^+ concentration immediately outside the squid axon membrane is still about 0.001 M larger than in the resting state.]

If AcCh would be released into the cleft, as postulated by the neurohumoral transmitter theory, and would not be hydrolyzed by AcCh-esterase of the pre- and postsynaptic membrane (an unlikely assumption), we estimate an average concentration of AcCh of about 10 μM. When AcCh is externally applied to the electroplax in this concentration, it has no effect on the membrane potential. Moreover, no AcCh is found outside the neuron unless potential enzyme inhibitors, such as physostigmine, are added to the perfusion fluid (8). On the other hand, the transient high concentration increase of K^+ ions may well affect the postsynaptic membrane and there induce AcCh to act on the receptor. This suggestion appears more likely than the inferred assumption that about a thousand (at most a few thousand) molecules of AcCh escaping the action of pre- and postsynaptic esterases can reach the postsynaptic receptors.

Movement of K^+ ions towards the postjunctional part of the synaptic cleft will not only be subject to a concentration gradient but also to the transient potential difference across the cleft, as long as the presynaptic nerve terminal remains positive during the action potential.

The effect of the approaching K^+ ions on the postsynaptic membrane may be strongly dependent on organization variations of the postjunctional part of the synapse. Due to invaginations, as for instance in the neuromuscular junctions and other structural modifications, there are ranges of the membrane that have different distances and different orientations to the presynaptic part. Chemical modifications, participation of neuroeffectors (like the catecholamines), and other processes within the synapse possibly give rise to excitatory as well as inhibitory properties. In the simplest case, the presynaptically released K^+ ions locally depolarize parts of the postsynaptic membrane. A great number of postsynaptic AcCh ions may thereby be induced to act on the receptors of many different BEUs. When only a few receptors per BEU are affected, local permeability changes will arise. As long as the cooperative number of receptors within one BEU is not involved in AcCh binding, there will be no action potential. The subthreshold effects of many BEUs, however, may accumulate and give rise to the appearance of the so-called synaptic or dendritic potentials. Thus, the basic mechanism for the initiation of nerve activity by mobilizing AcCh within the membranes of the synapse is suggested to be the same as in an axon.

8. Artificial Induction of Nerve Activity by AcCh. The translocation paths for AcCh: storage–receptor and receptor–esterase, may be artificially reproduced by external application of AcCh (or AcCh-like agents). This is, however, in a direct way, only possible in some very limited sections of the excitable membrane that are not protected by the lipid-rich Schwann cell or corresponding structural barriers (impervious to AcCh). It is well known that AcCh applied at nerve junctions produces depolarizing (as well as, in certain cases, hyperpolarizing) synaptic potentials (8).

AcCh has been shown to act on several axonal membranes (8). It is most remarkable that AcCh ions, applied in high concentration in the form of an electrophoretic pulse, lead to the generation of action potentials even in the conductive part of excitable membranes (B. Hamprecht, personal communication). Such behavior is expected when we recall Eq. **4**: external application of the potential-determining ions, in the form of a proper ion pulse, may lead to AcCh release in the form of a pulse. Thus, external AcCh-pulses may directly affect the above-threshold number of receptors in a BEU within the required time interval and cause action potentials.

In concluding this essentially programmatic essay, we summarize that, according to our unifying concept, the various expressions of nerve activity, such as action potentials, postjunctional potentials, and miniature potentials, do not reflect different mechanisms but are merely the result of amplification and accumulation processes based on the same elementary reactions. The proposed model accounts for the observed versatility in structure and electric response on the membrane level and may serve as a framework for investigating the molecular basis of the hierarchical organization and control mechanisms in the network of neurons that constitutes the brain.

We thank Professor Manfred Eigen for constructive discussions and suggestions instrumental for this paper. Thanks are also due to Prof. Werner Loewenstein for critical comments. The financial support by grants of the NSF (NSF-GB-31122X), of NIH (NS-03304) and by the gifts from the New York Heart Association, Inc., and the Sloan Foundation is gratefully acknowledged.

1. Katchalsky, A. (1967) in *The Neurosciences*, eds. Quarton, G. C., Melnechuk, T. & Schmitt, F. O. (The Rockefeller University Press, New York), pp. 326–343.
2. Tasaki, I. (1968) *Nerve Excitation* (C. C Thomas, Springfield, Ill.).
3. Katchalsky, A. & Curran, P. F. (1965) *Nonequilibrium Thermodynamics in Biophysics* (Harvard University Press); Plonsey, R. (1969) *Bioelectric Phenomena* (McGraw-Hill, New York).
4. Cole, K. S. (1965) *Physiol. Revs.* **45**, 340–379.
5. Hodgkin, A. L. (1964) *The Conduction of the Nervous Impulse* (C. C Thomas, Springfield, Ill.).
6. Abbott, B. C., Hill, A. V. & Howarth, J. V. (1958) *Proc. Roy. Soc. Ser. B.* **148**, 149–187; Howarth, J. V., Keynes, R. D. & Ritchie, J. M. (1968) *J. Physiol. (London)* **194**, 745–793.
7. Adam, G. (1970) in *Physical Principles of Biological Membranes*, eds. Snell, F., Wolken, J., Iverson, G. & Lam, J. (Gordon-Breach, New York), pp. 35–64.
8. Nachmansohn, D. (1970) *Science* **168**, 1059–1066; (1971) *Proc. Nat. Acad. Sci. USA* **68**, 3170–3174; (1969) *J. Gen. Physiol.* **54(2)** 187s–224s.
9. Kasai, M. & Changeux, J.-P. (1971) *J. Membrane Biol.* **6**, 24–57.
10. Cole, K. S. (1970) in *Physical Principles of Biological Membranes*, eds. Snell, F., Wolken, J., Iverson, G. & Lam, J. (Gordon-Breach, New York), pp. 1–15.
11. Katchalsky, A. (1964) in *Connective Tissue: Intercellular Macromolecules* (Little, Brown and Co., Boston), pp. 9–41.
12. Neumann, E. & Katchalsky, A. (1972) *Proc. Nat. Acad. Sci. USA* **69**, 993–997.
13. Neumann, E. & Rosenheck, K. (1972) *J. Membrane Biol.*, in press.
14. Hubbard, J. I. & Schmidt, R. F. (1963) *J. Physiol. (London)* **166**, 145–167.
15. Katz, B. & Miledi, R. (1965) *Proc. Roy. Soc. Ser. B.* **161**, 453–482.
16. Gage, P. W. & Moore, J. W. (1969) *Science* **166**, 510–512.
17. Feldberg, W. & Vartiainen, A. (1934) *J. Physiol. (London)* **83**, 103–128.
18. Eccles, J. C. (1935) *J. Physiol. (London)*, **84**, 50 p.
19. Frankenhaeuser, B. & Hodgkin, A. L. (1956) *J. Physiol. (London)* **131**, 341–376.