Ion channels in nerve membranes

The membranes of nerves, which play a key role in the conduction of impulses, are now believed to be traversed by protein channels with ion pathways opened and closed by the membrane electric field.

Gerald Ehrenstein

The molecular mechanisms underlying the message-carrying ability of nerves are not well understood. However, the experimental opportunities offered by the large-diameter "giant" nerve fibers of squid (see cover photo) and certain marine worms (see figure 1) have led to the discovery of significant facts about the origin of the nerve impulse.

The key to nerve conduction is the membrane, less than 100 Å thick, that separates the interior of the fiber from the rest of the animal. The nerve membrane is composed primarily of two types of macromolecules: protein and lipid (fat). During the passage of an impulse, sodium and potassium ions must cross this membrane. Evidence that I will describe in this article indicates that these ions cross the membrane by means of protein channels embedded in the membrane lipids, and that the conformation of the channels change under the influence of the membrane electric field.

Progress in nerve-membrane research is not only important intrinsically, but it also sheds light on other areas of membrane biophysics, including that of the utilization of metabolic energy.

Because of space limitations, this article provides only a summary of this active area of biophysics. The interested reader can find more detailed information in several books[1] and review articles.[2]

Signal transmission by nerves

Signalling in the nervous system takes place by the transmission of action potentials. Each action potential is a pulse about 100 mV in amplitude and 1 msec in duration, with a shape as shown in figure 2. Since the shapes of all action potentials are essentially the same, the information content of a signal resides in the sequence of action potentials and in the particular pathway it traverses.

The transmission line along which the action potentials travel is the nerve axon—a cylindrical structure that is part of a nerve cell. The function of the axon is to carry signals to the desired place—as much as several feet away in humans—at reasonable speed and without attenuation.

The axon consists basically of a cylinder of aqueous matter containing a high concentration of potassium, surrounded by a thin membrane. The membrane separates the contents, called the "axoplasm," from an external solution containing a high concentration of sodium. If the axon were a passive cable with a typical diameter of 2 microns, a transmitted signal would decrease to about 10^{-11} of its initial amplitude for one centimeter of path length. The axon, however, is an active unit, which employs a pumping mechanism to maintain ion gradients between inside and outside, and uses these gradients, in a manner we shall consider below, to maintain the amplitude of the action potentials.

Many axons are of the so called "myelinated" type; these are surrounded by an insulating myelin sheath over most of their length; at regular intervals the sheath contains breaks, called "nodes of Ranvier." A typical length between nodes is about 2 mm. In myelinated axons there is some decrement in signal amplitude over the internodal distance, but the signal is boosted back up to the standard action potential amplitude at each successive node. Other axons are nonmyelinated; these boost the action potential continuously to keep it at its standard amplitude. The advantage of myelination is that it results in a faster conduction velocity for a given axon diameter. The underlying mechanism for unattenuated signal transmission is essentially the same in both myelinated and nonmyelinated axons, and is the main subject of this article.

Properties of axon membranes

In the generation and transmission of action potentials, the axon membrane is the crucial element. The axoplasm can be replaced by a solution of potassium chloride or potassium fluoride without adversely affecting axonal performance for thousands of impulses, whereas even slight damage to the membrane seriously alters axonal properties.

The membrane regulates two kinds of ion transport, each of which is necessary for the axon to function:

- Active transport, required to maintain the ionic gradients across the membrane—in particular, the sodium and potassium gradients. This is accomplished by a mechanism that utilizes metabolic energy from the hydrolysis of adenosine triphosphate (ATP) to move ions across the membrane against their electrochemical gradients. As a result of this process, the axon interior has a high concentration of potassium and a low concentration of sodium as compared to the extracellular solution.
- The passive movement of sodium and potassium ions down their electrochemical gradients, in a manner determined by membrane potential. There is now a rather accurate phonomenological description of this passive transport, the

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PHOTOS: LEONARD BINSTOCK

Myxicola, a marine worm with a giant nerve fiber. The upper photograph shows a live specimen in a laboratory aquarium. The funnel, through which the animal ingests its food, is about 2 cm long. The bottom picture shows the results of a dissection in which the giant axon has been separated from the rest of the animal. In a voltage-clamp experiment the axon is cut out and mounted in a chamber with flowing seawater, and electrodes are inserted. Figure 1

Hodgkin–Huxley formulation.[1,2] It is based on experiments with axons in which temporal and spatial changes of membrane voltage are deliberately prevented. The method, called "voltage clamping," was first developed by Kenneth Cole.[1] A schematic diagram of a recent version of this method is shown in figure 3.

An important aspect of the type of voltage clamping shown in the figure is that the axon must be large enough to allow the insertion of a wire longitudinally into the nerve fiber to maintain the entire length of axoplasm at constant voltage and to act as a current electrode. The axons that have been used most are the giant axons of the squid and of a marine worm, myxicola, which are about a half millimeter in diameter. Figure 1 shows a live myxicola and one that has been dissected, with the giant axon prepared for experimentation.

Before presenting the detailed phenomenological description of membrane action, I would like to describe qualitatively how the action potential arises. A depolarization of the axon membrane (a voltage change making the inside more positive) causes an increase in the sodium conductance of the membrane; how this occurs will be considered below. Because of the high sodium concentration outside, this drives sodium ions into the axon, causing a further depolarization—and a further increase in sodium conductance. This positive feedback drives the membrane to the positive peak of the action potential. Subsequently, the voltage-dependent potassium conductance increases, giving rise to an outward flow of potassium ions, and driving the membrane back to its resting potential.

In the resting state the membrane voltage (the potential inside minus that outside, according to convention) is approximately −60 mV. When the membrane voltage is clamped to more positive voltages, the corresponding currents vary with time, as shown in figure 4a. On the basis of other experiments, in which external sodium ions were replaced with relatively impermeable ions, Alan Hodgkin and Andrew Huxley concluded in 1952 that the records of figure 4a represent two independent kinetic components of current—a transient component carried primarily by sodium ions, and a steady-state component, which develops more slowly and is carried primarily by potassium ions.

The peak transient currents and the steady-state currents are plotted as a function of membrane voltage in figure 4b. One confirmation of the two-component model is afforded by the value of V_{Na}, the "sodium-reversal potential," the voltage at which the peak transient current crosses the zero-current axis. In figure 4b, this is seen to be about +55 mV, in good agreement with the value predicted for sodium by the Nernst equation for monovalent cations,

$$V = \frac{RT}{F} \ln(a_o/a_i) \qquad (1)$$

Here R is the gas constant, T is the absolute temperature, F is Faraday's constant, and a_o and a_i are the external and internal sodium-ion activities; activity is the product of the activity coefficient (about 0.7 in this case) and the concentration.

The Hodgkin–Huxley equations describe the time course of the membrane current as a function of the membrane voltage and six auxiliary variables that are themselves empirical functions of membrane voltage. The rationale behind the equations is that each component of current is the product of a driving force and a conductance. The driving force is the difference between the actual membrane voltage and the appropriate equilibrium potential, and the conductance has a rather complex voltage dependence, described by three auxiliary variables, m, h, and n.

The next six equations are collectively known as the Hodgkin–Huxley equations:

$$I_i = I_L + I_{Na} + I_K \qquad (2)$$

where I_i is the total ionic current density;

I_L is the leakage current density, which is ohmic and usually small compared to the other components; I_{Na} is the transient component of current density (shown by the circles in figure 4), and I_K is the steady-state component of current density (shown by the squares in figure 4).

$$I_{Na} = g_{Na} m^3 h (V - V_{Na}) \qquad (3)$$

$$I_K = g_K n^4 (V - V_K) \qquad (4)$$

where g_{Na} is the maximum transient conductance (about 120 millisiemens/cm^2 for the squid axon) and g_K is the maximum steady-state conductance (about 40 millisiemens/cm^2 for the squid axon). The "siemens" is more familiarly known as the "mho."

The auxiliary variables m, h, and n are the solutions of the first-order equations

$$\frac{dm}{dt} = \frac{m_\infty(V) - m}{\tau_m(V)} \qquad (5)$$

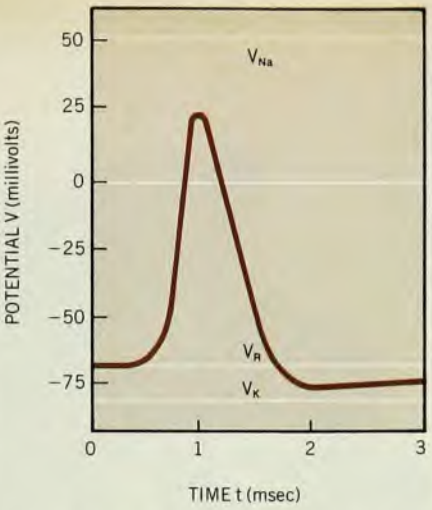
$$\frac{dh}{dt} = \frac{h_\infty(V) - h}{\tau_h(V)} \qquad (6)$$

$$\frac{dn}{dt} = \frac{n_\infty(V) - n}{\tau_n(V)} \qquad (7)$$

where $m_\infty(V)$, $\tau_m(V)$, $h_\infty(V)$, $\tau_h(V)$, $n_\infty(V)$ and $\tau_n(V)$ are voltage-dependent empirical parameters, the values of which are plotted in figure 5.

The Hodgkin–Huxley equations provide a good description of most of the experimental data on the electrical responses of axons. They also provide reasonable predictions for the time course of currents during voltage clamp, for the time course of membrane voltage during an action potential and for the durations of the absolute (no impulse possible) and relative (raised threshold for an impulse) refractory periods that follow an action potential. Nevertheless, there are a number of discrepancies between experiment and the Hodgkin–Huxley formulation. We will consider some of these below, in relation to more molecularly oriented models. For calculations of neurological behavior in nerve networks, the Hodgkin–Huxley equations are quite adequate. In fact, calculations on nerve networks are often made with simplified equations that are even less accurate than the Hodgkin–Huxley equations, but show the same qualitative features. An example of such simplified equations is Richard FitzHugh's model based on the Bonhoeffer–Van der Pol equations.[3]

Because the phenomenological equations described above are adequate for most neurological applications, the question may be raised as to the reason for seeking a more detailed, physical description. The answer lies primarily in the desire to understand the physical mechanisms behind the behavior of nerve-axon membranes. Furthermore, it has become increasingly apparent that membrane phenomena are crucial to a whole range of biological functions including oxidative phosphorylation, active transport and hormone regulation. The similarity in the overall membrane structure, from species to species and from cell to cell, gives us reason to expect that progress in one area of membrane behavior will lead to insight in other areas. The present search for a better understanding of the molecular mechanisms underlying nervous excitation is therefore as much related to membrane biophysics as it is to neurophysiology.



The action potential of a typical nerve. The white lines indicate the equilibrium potentials V_{Na} and V_K for sodium and potassium, and V_R, the normal resting potential. Figure 2

Evidence for ionic channels

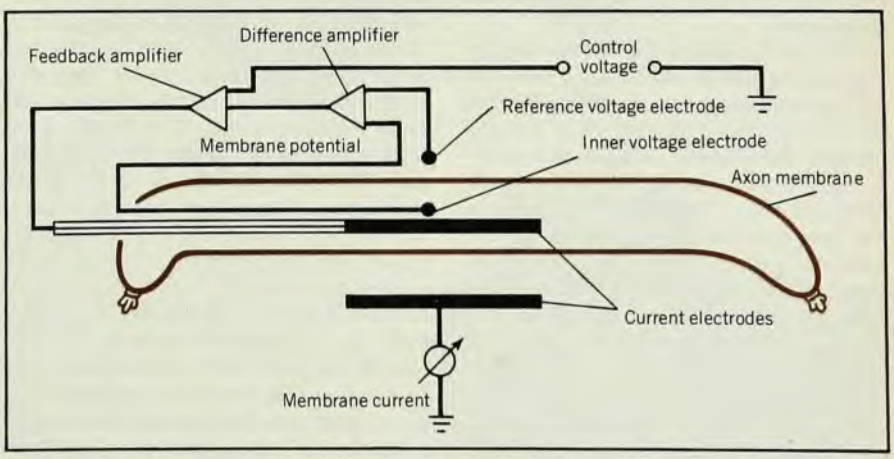
It is well established that the axon membrane (and biological membranes in general) has a lipid bilayer structure and that its dielectric constant is between two and three. The energy barrier presented by such a membrane to an ion of radius 1 Å in aqueous solution, about 46 kcal/mole, corresponds to a partition coefficient of the order of 10^{-35}, which implies an extremely low conductance.

In fact, synthetic lipid bilayers do have very low electrical conductance—about 10^{-8} siemens cm^{-2} for a 0.1 M salt solution. However, the conductances of biological membranes are 4–5 orders of magnitude larger in the resting state, with further increases during activity of 2–3 orders of magnitude.

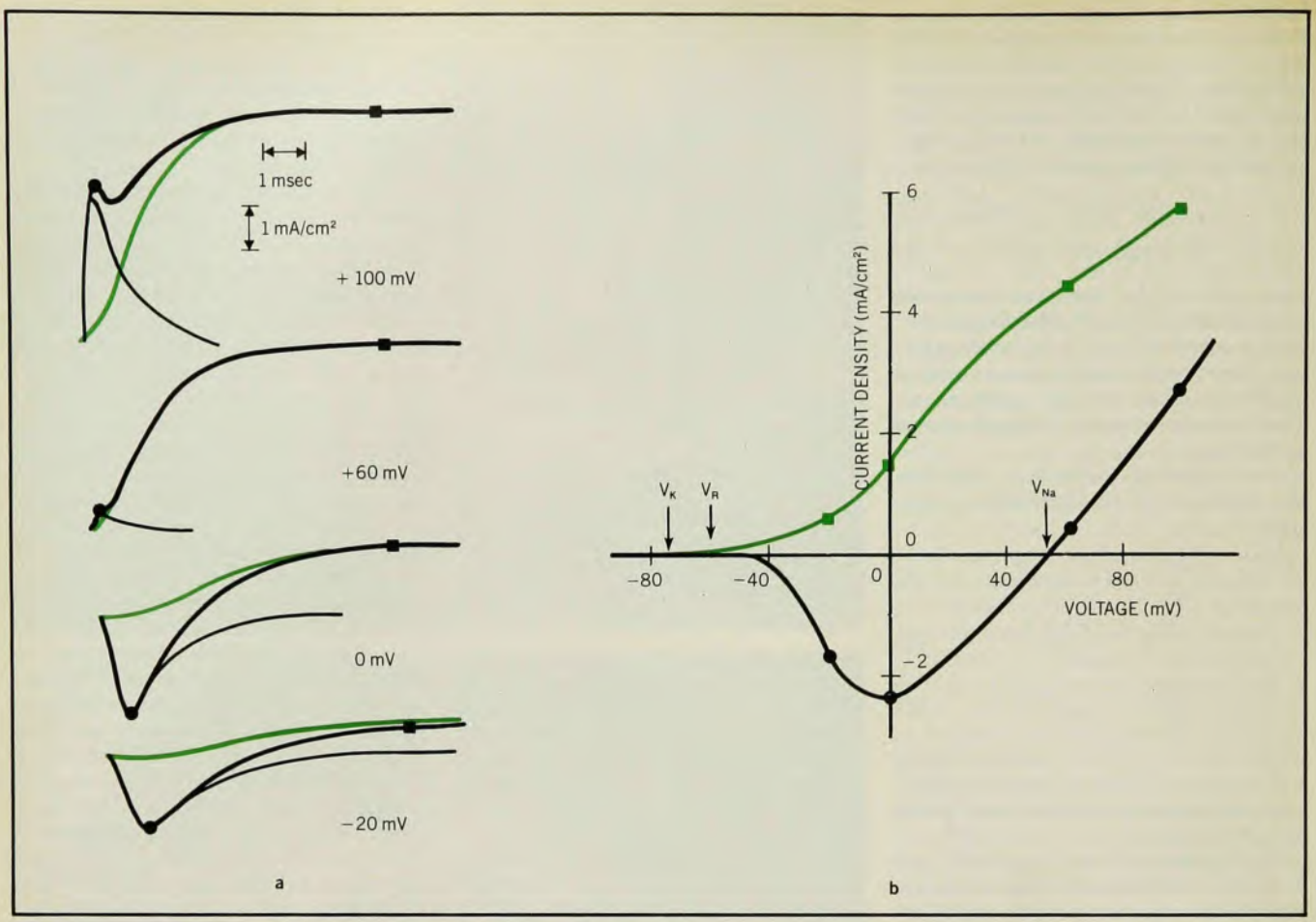
This relatively large flow of ions implies that there must be hydrophilic pathways in the membrane that can shield the ions from the lipid environment of the membrane. Two types of pathways are possible: carriers or channels. A carrier is a molecule with a hydrophilic interior that forms, with the ion, a complex with reasonable lipid solubility. A channel also has a hydrophilic interior and a hydrophobic exterior, but it is a fixed structure. The operational distinction is that a carrier moves with the ion and transports only one or a few ions across the membrane for each traversal of the membrane, whereas a channel does not move, and can provide a path for relatively many ions.

Both carrier and channel mechanisms have been convincingly demonstrated to exist in synthetic lipid bilayers.[4] Examples of carriers are valinomycin and monactin; examples of channels are EIM (excitability-inducing material extracted from the bacteria *Aerobacter cloacae*), alamethicin and gramicidin. All of these molecules (with the possible exception of EIM, the structure of which is not yet known) are polypeptides. As the title of this article suggests, there is now good evidence that channels provide the pathways by which ions cross axon membranes.

The main argument supporting the channel hypothesis is that the size of the smallest discrete unit of conductance in axons appears to be about 10^{-11} siemens, whereas the estimated maximum conductance of a single carrier molecule limited by translational diffusion is about 10^{-14} siemens, an estimate experimentally confirmed for valinomycin and monactin in lipid bilayers. The value for the elementary unit of conductance in axons



Schematic of an axon under voltage clamp. The voltage across the membrane (in color) is compared with a control voltage. Any potential difference drives current from the internal wire through the membrane (and the ammeter) in the direction that will reduce the difference to zero; this measures the current required to maintain a desired voltage. Figure 3



The decomposition of the membrane currents. The graphs in **a** show the time course of the currents through an axon membrane in response to changes in membrane potential from the resting potential (approximately −60 mV) to the potentials indicated in the figure. The thin black lines are the sodium, and the colored lines the potassium components. Current–voltage curves obtained from these transients are shown in **b**. The colored upper curve (squares) describes the steady-state component of current corresponding to the squares in the graphs on the left. The black curve (circles) describes the peak transient current, corresponding to the circles on the left. Figure 4

is based on several different types of indirect experiments, the assumptions of which are difficult to evaluate. Nevertheless, all these experimental methods give estimates between 10^{-10} and 10^{-12} siemens for the elementary unit of conductance. Taken together, these experiments give strong support to the channel hypothesis.

The ideal method for measuring the elementary unit of conductance would be to voltage-clamp an axon membrane containing relatively few channels and observe the discrete changes of conductance that occur as individual channels open and close. This type of experiment has been successful in measuring the elementary unit of conductance for EIM, alamethicin and gramicidin channels in lipid bilayers,[5] and is being actively pursued for biological membranes. It has not yet proven successful for axon channels.

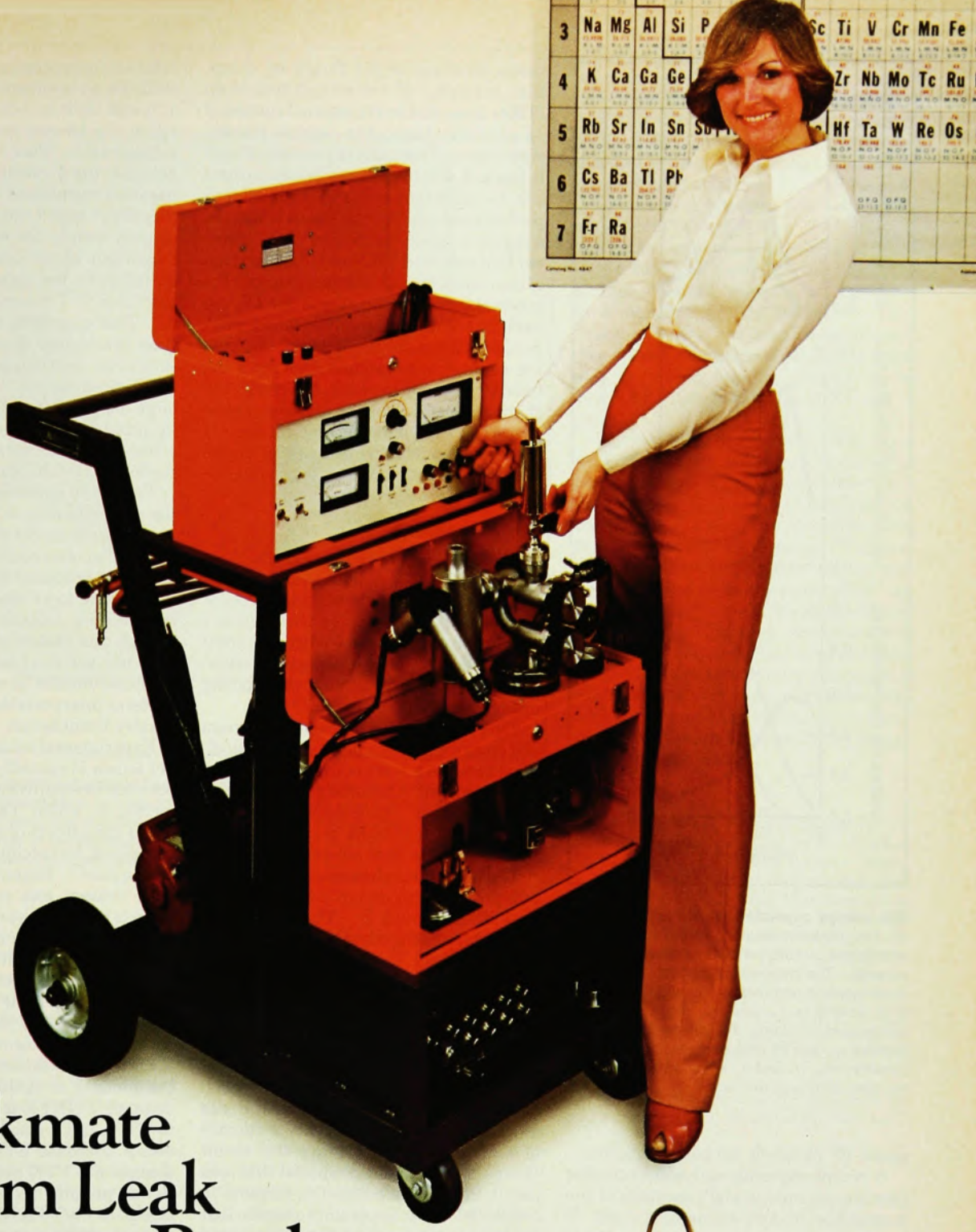
Examination of the power spectrum of membrane current noise during voltage clamp has made it possible, however, to estimate the elementary unit of conductance from the overall effect of large numbers of discrete conductance changes. A simple type of two-state model, in which each elementary conductance unit is either on or off and the voltage dependence of the conductance resides in the voltage-dependent probability that the conductance is on, leads to a power spectrum of the form:

$$S(f) = S(0)/[1 + (f/f_{1/2})^2] \qquad (8)$$

where both $S(0)$ and $f_{1/2}$ are, in general, voltage dependent. A number of other physically reasonable models also give power spectra that are very similar to the form of this equation. The amplitude of $S(0)$ is related to the size of the elementary unit of conductance. For a given mean value of membrane current, a larger size for the elementary unit of conductance (and hence fewer units) results in more current noise. Although the exact relation between $S(0)$ and the elementary unit of conductance depends on the model, estimates of the elementary unit of conductance based on experimental noise spectra fall into a narrow range for a number of reasonable models. They give[6] about 4×10^{-12} siemens for the sodium channel and about 10^{-11} siemens for the potassium channel.

H. C. Luttgau made another type of fluctuation measurement in 1958. He applied subthreshold current stimulation to a node of Ranvier, and observed that the voltage responses were not distributed randomly about the mean but appeared to cluster near discrete voltages approximately 1 mV apart. If the transient opening of individual sodium channels caused the discreteness in voltage response, the sodium channel conductance would be about 10^{-10} siemens.

Another approach to determining the elementary unit of conductance has been to make use of the drug tetrodotoxin (TTX), which is known to decrease the transient conductance, presumably by blocking sodium channels. The density of the putative sodium channels has been estimated by measurement of the amount of TTX required to block the channels and independently by measurement of the kinetics of blockage. From the density of sodium channels, the conductance of a single channel can be determined simply for those preparations where the total sodium conductance is known. The value for elementary conductance determined in this way is about 10^{-11} siemens, and the density of sodium channels is



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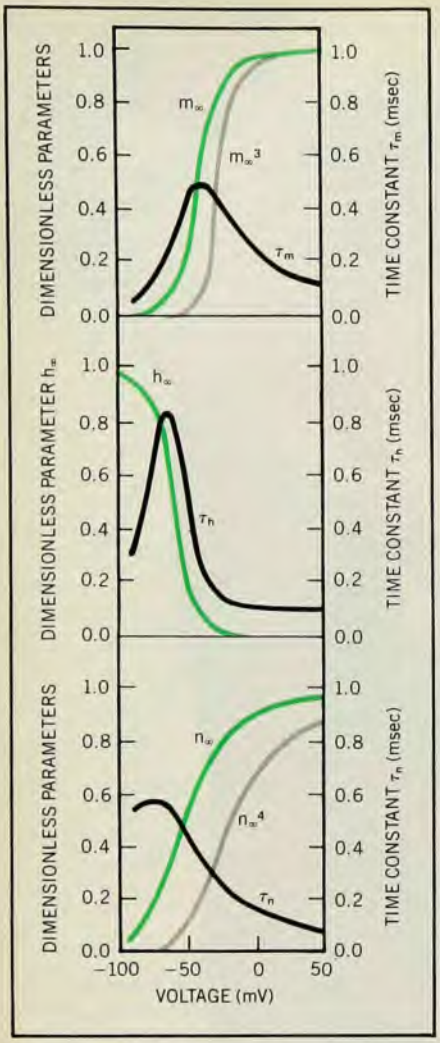


The voltage dependences of the parameters in the Hodgkin–Huxley formulation. The membrane current consists of two main components: The transient component contains the dimensionless parameters m_∞ and h_∞ and their time constants τ_m and τ_h; the steady-state component contains the dimensionless parameter n_∞ and its time constant τ_n. The parameters m_∞, h_∞ and n_∞ are shown in color and the time constants in black. Figure 5

about 10^2 channels per square micron.

A recent experimental approach that provides an independent estimate of the magnitude of the elementary unit of conductance is the measurement of "gating currents." These are currents caused by the movement of charged portions of the structures, called "gates," responsible for changing the voltage-dependent ionic current.[7] Gating-current measurements provide an estimate of the equivalent gating-charge density crossing the membrane. With a reasonable estimate of the number of gating charges per channel, the channel density can be calculated. The value for the elementary unit of conductance for the transient sodium process obtained in this way is also about 10^{-11} siemens.

Another type of evidence favoring channels over carriers is the unidirectional blockage of ion fluxes by drugs. For example, if tetraethylammonium (TEA) is applied to the internal surface of an axon membrane, the outward steady-state current (normally of potassium ions) is blocked, but inward potassium current can be maintained. The channel hypothesis can explain this result if there is a region at the inner surface of the channel that can bind TEA and if the portion of the channel between this region and the external surface is too narrow for TEA to pass through. Internally applied TEA would then bind to the channel, blocking the passage of potassium ions from the inside; potassium ions entering the channel from the outside, however, could sweep the TEA from the binding site. It is difficult to conceive of a carrier model that is consistent with this type of unidirectional blocking.

The ion channels

We have seen that several kinds of experimental evidence lead to the conclusion that ions cross the axon membrane by means of channels. In this section I will summarize some of what is known about the sodium and potassium channels—particularly about the gating mechanisms.

Figure 6 is a rather speculative diagram of a cross section of a potassium channel. It synthesizes a body of experimental information, but it is certainly not unique in that respect. Its purpose is more to clarify what questions are being asked than to provide final answers.

The potassium-channel model consists of a protein with an ion pathway (pore) extending through it. The pore has a hydrophilic lining containing at least two binding sites. This view of the pore is based on a suggestion Hodgkin and Richard Keynes made to explain their potassium flux measurements: They found that the ratio of potassium influx to potassium efflux varies with the external potassium concentration much more than would be expected if the ions moved through a channel independently of each other. To explain this result Hodgkin and Keynes proposed that ions pass through the potassium channel in single file. (It is important to realize that in these experiments efflux is measured with radioactive potassium only inside the axon, and influx is measured with radioactive potassium only outside the axon.)

The single-file restriction makes the measured flux ratio more dependent on the inside-to-outside concentration ratio as the number of elements in the file increases.[8] The experimental data can be fit with 2–3 ions in file. Comparable experiments on sodium fluxes do not indicate this type of long-pore behavior.

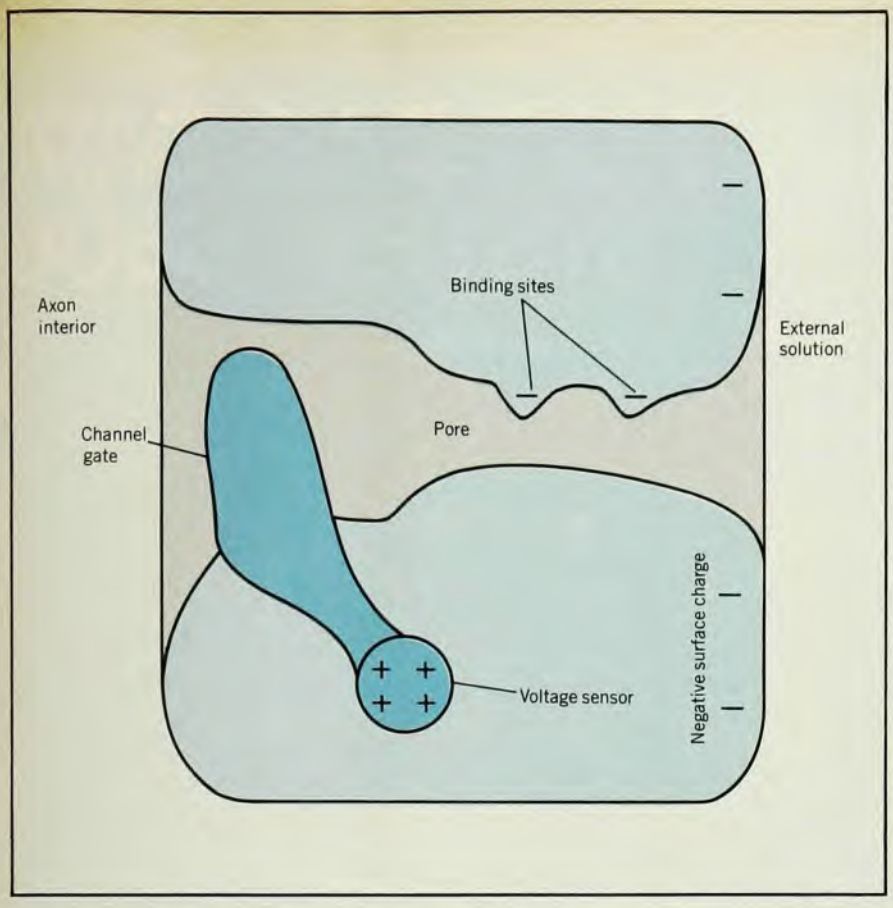
In figure 6, negative charges are indicated on the external surface opposite the voltage sensor because of considerable experimental evidence that changes in the external concentration of divalent cations shift the phenomenological curves of m, n, h and their associated time constants τ_m, τ_n, τ_h (shown in figure 5) along the voltage axis. This result would be expected if the divalent cations neutralized negative membrane surface charge and thereby changed the electric field that is actually seen by the voltage sensors. The magnitude of surface charge density required to fit the experimental data is in the range of 1 electronic charge per 200 Å^2. This is an order of magnitude higher than the charge density that was measured over large areas of cell membranes by electrophoresis. It is likely that the large negative charge density is a highly localized phenomenon, caused by one or a few negative charges on the external surface of the channel protein.

The actual molecular events underlying the gating process are not known, but it appears reasonable that the voltage-sensor portion of a membrane-bound protein translates or rotates within the membrane in response to an electric field, and that this motion triggers a conformational change that blocks or unblocks the pore. It is not yet clear whether this process involves subunits, as would be required by a literal interpretation of the Hodgkin–Huxley formulation.

Experimental evidence that the channel sensor is spatially removed from the pore has been provided by recent experiments, in which Ted Begenisich compared the effects of surface-charge neutralization by calcium on the sensor and on the pore.[9] Begenisich found that, for both sodium and potassium channels, there is much less surface charge near the channel pore than near the sensor.

For the sodium channel, there is additional evidence about the surface charge densities. Richard Henderson, J. M. Ritchie and Gary Strichartz measured equilibrium constants for the binding of TTX to the sodium channel, and also for the binding of saxitoxin (STX) to that channel.[10] (STX is similar in structure to TTX, and it also blocks sodium channels.) Because STX has two positive charges and TTX has one positive charge, it was possible to estimate the surface charge at the TTX binding site (the pore) from the difference in effects of calcium-concentration changes on the binding of the two drugs. This surface charge was found to be several times smaller than the effective negative surface charge density at the sensor.

A funnel-like shape for the open pore is necessary to fit experimental data on the effects of tetraethylammonium (TEA) and some of its derivatives. When TEA is applied internally, outward potassium current is blocked, but inward potassium is not. Furthermore, the block can be relieved by increasing the flux of inward potassium. If TEA ions lodged in the mouth of the pore, they would be electrostatically repelled by inward-moving



The cross section of a model potassium channel, postulated to traverse the axon membrane. The gate is shown in its closed position; potassium ions can not cross the membrane. When the inside potential of the axon is made less negative, the electric field at the voltage sensor changes, causing a conformational change of the channel protein. This opens the channel gate, providing a path for potassium ions to cross the membrane. Figure 6

potassium ions, but unaffected by outward-moving potassium ions—in agreement with the experimental results. Since the TEA ion is about 8 Å in diameter, the diameter of the mouth must be at least 8 Å.

The reason for placing the gate at the inside surface of the pore is the evidence that TEA can be trapped in the pore when the gates are closed.

The best information relating to the diameter of the narrow part of the pore is the relative permeability of various ions. Ions with crystal diameters between about 2.6 and 3.0 (potassium and ammonium, for example) permeate the membrane, whereas smaller ions (such as sodium) and larger ions (such as cesium) do not. To explain this it has been suggested that the narrow part of the pore may have a hydrophilic lining and is sufficiently flexible to adjust its diameter between 2.6 and 3.0 Å. Ions in that size range would have energetically favorable interactions with the pore lining, but larger or smaller ions would not.

For sodium channels, permeability studies have been made on a much larger group of ions. Bertil Hille found[11] that a number of organic cations permeate through the sodium pore, and that all of them have an effective cross section no larger than 3 × 5 Å. A particularly interesting comparison is afforded by hydroxylammonium and methylammonium. Hydroxylammonium (H_3N^+–OH) is rather permeable, whereas methylammonium (H_3N^+–CH_3), which is about the same size (approximately 4.5 × 3.8 × 3.8 Å), is quite impermeable. To explain this, Hille suggested that there are oxygen atoms lining the sodium pore, and that the hydroxyl hydrogen of the hydroxylammonium ion can form hydrogen bonds with these oxygen atoms. In Hille's model, this effectively reduces the ion diameter so that hydroxylammonium can pass through a 3 × 5 Å pore. The proposal that oxygen atoms line a pore is very reasonable in view of the fact that oxygen atoms line the pore of gramicidin A, an antibiotic that forms cation-permeable pores in lipid bilayers.

The final question I will consider is the relation between the m and h gates of the sodium channel. According to the Hodgkin–Huxley formulation, the m and h gating processes are independent of each other. There is now evidence that this view must be modified. The gating currents that have been observed relate primarily to the m gate. If the m and h gates were independent, the gating current during a test pulse would depend only upon the value of the parameter m at the start of the pulse, and not at all on h. In fact, Clay Armstrong and Francisco Bezanilla have shown that the gating current is proportional to h. This indicates that the m and h gates are not independent of each other.

Further evidence for coupling of the m and h gates is afforded by the studies on sodium-channel kinetics of Lawrence Goldman and Charles Schauf.[12] They found that, at some membrane voltages, two different methods of measuring h and τ_h give different results, contrary to the prediction for independent m and h gates.

The emerging picture is that ion channels are complex protein molecules with conformations that provide a complete path between the inside and outside of the membrane, but that only certain ions can readily permeate these pores. The protein probably has a charged region, spatially removed from the pore, which can move in response to a changing electric field, thereby causing a conformational change that somehow blocks or unblocks the pore. The elucidation of this putative conformational change will resolve the most important remaining question concerning the mechanism of signal propagation in nerve cells.

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