INTERACTIONS BETWEEN INTRINSIC MEMBRANE PROTEIN AND ELECTRIC FIELD

AN APPROACH TO STUDYING NERVE EXCITABILITY

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ABSTRACT We have approached the problem of nerve excitability through three questions: (*a*) What is the diagram for a channel? That is, what conformational states can the protein assume, and what transitions between these conformations are permitted? (*b*) What is the channel conductance associated with each conformation the channel can assume? (*c*) How do the rates for conformational transition depend upon membrane potential? These three questions arise from a standard statistical mechanical treatment of a nerve membrane containing several classes of identical, independent channels. Gating of channels, in this view, is associated with conformational changes of the channel protein, and it is assumed these conformations are distinct. The precise formulation of these questions is presented in terms of the theoretical treatment, and the approaches we have taken to answer the questions are indicated. Our present results indicate: Transition rates should depend exponentially on membrane potential over a limited voltage range, but probably will show a more complex dependence for extremes of the range; channels probably can take on only two conductances, open and shut, but more complicated situations are not entirely excluded; the diagram for a channel cannot be determined from standard voltage clamp data alone, but by studying gating currents and conductance fluctuations, it should be possible to select between alternative plausible physical mechanisms.

Ever since the publication of the Hodgkin and Huxley (1952) analysis of nerve excitability 25 years ago, a clear goal of membrane biophysics has been to give a physical account of the phenomena described by their equations. One important step in approaching this goal is to decide what questions must be answered to provide such an account. Even formulating these questions precisely is, however, a difficult task and depends on the theoretical framework chosen. My goal here is to describe the framework within which my laboratory has approached the problem of nerve excitability, to give a statement of the questions that must—within this theoretical context—be answered, and to indicate some experimental strategies we have adopted in an attempt to find answers.

Our starting place is to assume that separate channels exist for the various current components (Na, K, etc.) revealed in voltage clamp experiments, and to suppose that

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channels of a given type are identical and independent of each other and of other types. Further, we postulate that gating occurs by conformational changes in the channel protein, and that a given type of channel can assume only specific and distinct conformational states. From these starting assumptions a standard statistical, mechanical formulation of the problem defines the questions that experiments must be designed to answer. The treatment given here is a more complete version of the one indicated by Magleby and Stevens (1972).

The discussion that follows will start with a presentation of the differential equations appropriate to describe channel behavior. I will then focus on the problem of transition rates from one state to another, and particularly on the voltage dependence of these rates. The treatment of transition rates is formulated in terms of protein-membrane field interactions, and uses techniques similar to those standard in statistical theories of dielectric properties. Finally I will summarize the questions that arise from this formulation of the problem and indicate some of our experimental approaches to them.

THE MASTER EQUATION APPROACH

Because channels of a given type have been assumed to be independent and identical, we need treat only a single channel; the properties of an ensemble of such channels is then related simply to those of the single channel. A standard way to treat non-equilibrium behavior is through a master equation (see Zwanzig, 1964), and this approach becomes particularly simple for a system such as a channel with only a relatively small number of distinct conformations (see Oppenheim et al. 1967).

Suppose a channel can have n conformations: $1, \ldots, k, \ldots n$, and let $P_k(t)$ be the probability of finding the k^{th} conformation at time t. For a membrane with N channels, we would measure the average conductance $g(t)$ given by

$$g(t) = N \sum_{k=1}^{n} \gamma_k P_k(t) \tag{1}$$

where γ_K is the average conductance of a channel in conformation k; the quantity $\Sigma_k \gamma_k P_k(t)$ is just the average conductance of one channel. To calculate a membrane conductance as a function of time, then, we must know the probability $P_k(t)$ of each conformation and the associated conductance γ_k.

The probability of each conformation is governed by a master equation. To write the master equation for a channel we must know first, what conformations the channel can have and which transitions between conformations are possible, and second, at what rates the permitted transitions occur and how these rates depend on voltage. The master equation that describes the behavior of our n-conformation channel is then

$$\mathrm{d}P_k(t)/\mathrm{d}t = \sum_j \alpha_{jk} P_j(t) - \sum_j \alpha_{kj} P_k(t), \tag{2}$$

where α_{jk} is the transition rate from conformation j to conformation k, and the summations above include all permitted transitions. Eqs. 1 and 2, together with information about the values for rate constants α_{jk}, give a complete description of channel behavior.

VOLTAGE DEPENDENCE OF TRANSITION RATES

The existence of "distinct conformations" has been assumed, which means, in this context, that various principal conformations of the channel-gate can be identified and that the channel must pass through a higher energy and infrequently occurring transitional conformation to go from one conformation to another. In this situation, the rate constants $\alpha_{jk}(V)$ as a function of membrane potential V are given by absolute rate theory (see for example, Prigogine and Bak, 1959):

$$\alpha_{jk}(V) = \nu e^{-\beta U_{jk}(V)} \tag{3}$$

where ν is an attempt rate, $U_{jk}(V)$ is the free energy difference between the j^{th} conformation and the transition conformation that separates j from k, and $\beta = 1/kT$ (k is Boltzmann constant, T is temperature). To understand how voltage influences $\alpha_{jk}(V)$, we must know $U_{jk}(V)$, and this quantity can in turn be calculated if the general dependence on voltage of the free energy of a conformation is known. We turn now to a study of the free energy $U_k(V)$ of the k^{th} conformation (either principal or transitional). The approach to be used is basically similar to that first adopted by Kirkwood (1939) in his treatment of the dielectric constant; specifically, the voltage dependence of $U_k(V)$ will be expressed in terms of the energy $U_k(0)$ in the absence of applied field. In this way the complicated molecular interactions that occur can be separated from the effect of applied electric fields.

The free energy of the k^{th} conformation is calculated, in the standard way, from the partition function Z_k for conformation k according to $U_k = -\partial \ln Z_k/\partial\beta$. The conformation k is made up of a number of states with somewhat different arrangements of the constituent atoms. For example, the channel might be in conformation k with or without a H^+ dissociated from some particular carbosylic acid group. Call each arrangement of atoms, consistent with the integrity of channel structure, a state of the channel, and designate energy of the r^{th} state by $w_r(V)$. The partition function is then calculated from the energies $w_r(V)$ of the states that make up the k^{th} conformation in the standard way,

$$Z_k = \sum_{r\in\{k\}} e^{-\beta w_r(V)} \tag{4}$$

where the notation "$r \in \{k\}$" indicates that the sum extends over all states that constitute the conformation k. Note that "distinct conformations" are defined so that the barrier separating them is appreciably larger than the barriers between the states that constitute any particular conformation. The next step in obtaining an expression for the voltage dependence of U_k, and hence of α_{jk}, is to separate the energy $w_r(V)$ in terms of the energy of the r^{th} configuration in the absence of an applied field and the contributions due to the presence of the field.

The r^{th} state consists of a particular arrangement of atoms which have an energy $w_r(0)$ for a zero membrane potential. The energy includes not only such factors as strain in bonds and hydrophobic interactions, but also electrical interactions between the various charges and dipoles and the reaction field they cause in the environment surrounding the channel. As the same configuration r is maintained and the field V/a (V is membrane potential, a is membrane thickness) is superimposed on the existing fields deriving from the arrangement of atoms, the energy of the r^{th} configuration changes by field-charge and field-dipole interactions and by the work of distortion polarization of atoms and bonds. The energy due to the presence of the field is thus reflected in three terms that add to the zero-voltage energy:

$$w_r(V) = w_r(0) - \frac{V}{a} \sum_{s \in \{k\}} q_{rs} f_{rs} - \frac{V}{a} \sum_{s \in \{k\}} \mu_{rs} \cdot \mathbf{n} - \frac{V^2}{2a^2} b_r, \tag{5}$$

where: $-(V/a) \sum_{s \in \{k\}} q_{rs} f_{rs}$ is the energy due to charge-field interaction, with q_{rs} the s^{th} charge that occurs in the r^{th} state, f_{rs} the distance through the membrane that the s^{th} charge has in state r, and with the sum running over all charges for conformation k; $-V/a \sum_{s \in \{k\}} \mu_{rs} \cdot \mathbf{n}$ is the energy due to dipole-field interactions, with μ_{rs} the dipole moment of the s^{th} dipole in the r^{th} state, $\mathbf{n}$ the unit vector normal to the membrane plane, and with the sum extending over all dipoles in conformation k; $-(V^2/2a^2) \cdot b_r$ is the energy due to distortion polarization with the simplyfing (but not essential) assumption that polarization is proportional to the applied field, the proportionality constant being b_r. This term for distortion polarization takes into account the dielectric properties of the channel and surrounding lipids that remain when all atoms are constrained to remain in the positions of the r^{th} state so that polarization by fixed dipole orientation is excluded (see for example, Debye's 1929 discussion of contributions to polarizability of a material.)

It should be emphasized that the applied field above is constant, but the actual field within the membrane doubtless varies wildly from point to point. Even in the absence of applied field (i.e., $V = 0$), there would be, within the channel structure, a very complicated field arising from the particular arrangement of changes, dipoles, etc. that constituted the r^{th} state. Nor would applying a constant external field necessarily just add a constant to the existing intrinsic field, because the distortion polarization would not be uniform through the membrane structures. Nevertheless, the energy contributions in Eq. 5 use only the applied field because of the linearity of electrostatics and the use of Kirkwood's method for expressing energies with an applied field present in terms of the energy of the same configuration of atoms with no field applied.

The task now is to make appropriate approximations that simplify Eq. 5. The first step is to simplify notation by defining the quantity $x_r(k)$ for the r^{th} state of conformation k by the equation

$$x_r(k) = \sum_{s \in \{k\}} q_{rs} f_{rs} + \sum_{s \in \{k\}} \mu_{rs} \cdot \mathbf{n}. \tag{6}$$

With this notation Eq. 5 is written

$$w_r(V) = w_r(0) - (V/a)x_r(k) - (V^2/2a^2)b_r.$$

The sums that make up $x_r(k)$ in Eq. 6 can each be divided into parts: one part that depends on both r and k, one part that depends upon k but not on r (as long as r is a state that is part of conformation k), and a third part that depends on neither k nor r. Physically this means that certain charges and dipoles have the same magnitude and position for all states of the channel (or at least all states with low enough energy to occur with appreciable frequency), others are the same for all states of one or more conformations but not for all conformations, and others have different values for various states that constitute one conformation. For example, a particular dipole might (*a*) have the same moment for every state the channel could assume; (*b*) have the same moment for all states of (say) an open conformation but a zero moment for all other states of the channel; or (*c*) have somewhat different values for various states of a particular conformation. This dipole would contribute to: the sum independent of r and k for (*a*), the sum that depends on k but not r for (*b*), and the sum that depends on r (and k) for (*c*). Rewrite Eq. 6 to divide $x_r(k)$ into the parts that depend on r and on k, and do not, for simplicity, include the component independent of both:

$$x_r(k) = m_k + m_r(k). \tag{7}$$

Here m_k is the part of the sums in Eq. 6 that has the same value for each of the states r that make up the conformation k, and $m_r(k)$ arises from contributions whose value varies with the r states. In an analogous way, replace the distortion polarization term $b_r(k)$ by the sum $b_k + b_r(k)$ and do not write the component that has the same value for all states in all conformations. The energy of the r^{th} state of conformation k can now be expressed

$$w_r(V) = w_r(0) - (V/a)[m_k + m_r(k) + (V/2a)(b_k + b_r(k))]. \tag{8}$$

Because only energy differences between various conformations at a fixed voltage will be important for the following discussion, omission of quantities common to the energies of all states (of all conformations) will not affect the final results.

The partition function for the k^{th} conformation is

$$\begin{aligned} Z_k &= \sum_{r \in \{k\}} \exp\left\{-\beta\left[w_r(0) - \frac{V}{a}\left(m_k + m_r(k) + \frac{V}{2a}(b_k + b_r(k))\right)\right]\right\}, \\ &= \exp\left[\beta\frac{V}{a}\left(m_k + \frac{V}{2a}b_k\right)\right] \sum_{r \in \{k\}} \exp\left\{-\beta\left[w_r(0) - \frac{V}{a}\left(m_r(k) + \frac{V}{2a}b_r(k)\right)\right]\right\}. \end{aligned} \tag{9}$$

From this partition function we can, using the relation $U_k = -\partial Z_k/\partial\beta$, calculate the free energy of any conformation as a function of applied field. Eq. 3 can then be used to obtain the voltage dependence of transition rates between conformations. Because Eq. 9 is still rather complicated, we turn now to some further simplifications.

The Low Field Limit

If the energies associated with the membrane potential are not too large, we may follow the procedure, standard since Debye (1929), of expanding about $V = 0$ the voltage-dependent part of the exponential that contains the quantities $m_r(k)$ and $b_r(k)$ and retaining only the first two terms of the expansion. This low-field approximation gives the expression

$$\ln Z_k(V) \approx \beta\frac{V}{a}\left(m_k + \frac{V}{2a}b_k\right) + \ln\sum_{r\in\{k\}} e^{-\beta w_r(0)}\left(1 + \frac{\beta V}{a}m_r(k) + \frac{\beta V^2}{a^2}b_r(k)\right). \quad (10)$$

The free energy of the k^{th} conformation is then approximately

$$\begin{aligned} U_k(V) = -\frac{\partial \ln Z_k(V)}{\partial\beta} &\approx U_k(0) - \frac{V}{a}m_k - \frac{V^2}{2a^2}b_k \\ &- \frac{V}{aZ_k(0)}\sum_{r\in\{k\}} m_r(k)e^{-\beta w_r(0)} \\ &- \frac{V^2}{2a^2Z_k(0)}\sum_{r\in\{k\}} b_r(k)e^{-\beta w_r(0)} \end{aligned} \quad (11)$$

Define the effective dipole moment M_k by

$$M_k = m_k + \frac{1}{Z_k(0)}\sum m_r(k)e^{-\beta w_r(0)} \quad (12)$$

and the total distortion polarizability

$$B_k = b_k + \frac{1}{Z_k(0)}\sum b_r(k)e^{-\beta w_r(0)} \quad (13)$$

The free energy of conformation k is then

$$U_k(V) = U_k(0) - (V/a)M_k - (V^2/2a^2)B_k. \quad (14)$$

The term B_k reflects differences in distortion polarization that occur between various conformations. The contribution of B_k should be small compared to other terms, however, because the distortion polarizability of atoms and bonds should not be sufficiently anisotropic to produce energy contributions from this source comparable to those from fixed charges and dipoles (see, O'Konski et al, 1959; LeFèvre, 1965). The energy of the k^{th} conformation, then, should be of the form

$$U_k(V) = U_k(0) - (V/a)M_k. \tag{15}$$

The transition rate from conformation j to k depends exponentially on the difference between two energies of this form so

$$\alpha_{jk}(V) = \nu \exp\left[-\beta\left(U_{jk}(0) - \frac{V}{a} M_{jk}\right)\right], \tag{16}$$

where the double subscripts indicate appropriate differences between quantities subscripted (e.g., $U_{jk}(0) = U^*_{jk}(0) - U_j(0)$, and $U^*_{jk}(0)$ is the energy with $V = 0$ of the transitional conformation going from j to k). Presumably the largest contribution to M_{jk} is the alteration in number of charges on the protein that accompanies conformational changes (e.g. Schlecht, 1969; French and Hammes, 1965; Giannini et al., 1975).

Approximations outside the Low Field Limit

If the low-field approximation is not valid, the energy of the k^{th} conformation is (again neglecting distortion polarization)

$$U_k(V) = U_k(0) - \frac{V}{a} m_k - \frac{V^2}{2a^2} b_k - \frac{\partial}{\partial \beta} \ln \sum_{r \in \{k\}} \exp\left\{-\beta\left(w_r(0) - \frac{V}{a} m_r(k)\right)\right\}. \tag{17}$$

The last term on the right of this expression is, in general, quite complicated. A plausible simplification can result, however, by considering which contributions are likely to dominate the energy $w_r(V)$ of a state.

The quantity $m_r(k)$ in Eq. 17 contains contributions from bond and group dipoles and from the presence of charges that experience a fraction of the membrane potential. Bond and group dipole moments are on the order of $1D$ (see, for example, Hill et al., 1969), which means that a single bond can provide, in a 100 kV/cm field, energies that amount to only about 1% of kT. Again, protein structure suggests that significant changes in energy due to differences in bond dipoles between various states of a single conformation cannot be large. One electronic charge moving about 25 Å in a 100 kV/cm field changes energy by $1kT$ unit. If the channel protein had a dissociable charge located within a membrane crevice, and if both the associated and dissociated states of the charge occur with the channel in conformation k, then the term $(V/a)m_r(k)$ could be comparable to kT for two states of a single conformation. In fact the only appreciable contributions to $(V/a)m_r(k)$ seem likely to arise through changes in the number of charges due to voltage driven association or dissociation.

Once membrane potential V is too large for the low-field approximation to hold with sufficient accuracy, the natural procedure is to terminate the power series expansion that led to Eq. 10 after the V^2 term rather than after the V term. Eq. 15 would then be changed into

$$U_k(V) \approx U_k(0) - (V/a)m_k - (V^2/2a^2)p_k, \tag{18}$$

where p_k is the contribution from terms in V^2. Although this equation would hold over a somewhat larger voltage range than Eq. 15, the approximation might still not be adequate in all instances.

For cases to which this approximation applies, the transition rates would be

$$\alpha_{jk} = \nu \exp\left\{-\beta\left(U_{jk}(0) - \frac{V}{a}m_k - \frac{V^2}{2a^2}p_k\right)\right\}, \tag{19}$$

where the double subscripts again indicate the result of taking the difference between the appropriate two terms of the form given in Eq. 18.

To examine the situation in which the above approximation is inadequate, suppose that the sum on the right of Eq. 17 contains only two terms corresponding to a single electronic charge being bound or dissociated. In this case, the sum reduces to

$$\sum e^{-\beta(w_r(0) - (V/a)m_r(k))} = e^{-\beta U_0} + e^{-\beta(U_1 - qfV/a)},$$

where U_0 is the energy with the charge bound to its site, U_1 is the energy when the charge is dissociated with zero membrane potential, q is the charge, and f the distance through the membrane field that the charge moves when it dissociates. The free energy $\tilde{U}_k(V)$ of the k^{th} conformation now becomes for this situation

$$\tilde{U}_k(V) = U_k(0) - (V/a)m_k + \frac{U_0 + (U_1 - qfV/a)\exp\{-\beta(U_1 - U_0 - qfV/a)\}}{1 + \exp\{-\beta(U_1 - U_0 - qfV/a)\}}. \tag{20}$$

The simplicity of Eq. 16 and 19 is lost here, and the expression becomes still more complicated if the above approach is generalized to include cases with more than only two terms in the sum. Nevertheless, Eq. 20 might well be expected to be a useful approximation when a single dissociable charge dominates the sum.

QUESTIONS TO BE ANSWERED

The equations developed above serve to define the three main questions my laboratory uses to approach nerve excitability. (*a*) From Eq. 2: What is the diagram for a channel? That is, how many principle conformations are there, and between which conformations are transitions permitted? (*b*) From Eq. 1: What is the conductance γ_k associated with each conformation k? (*c*) From Eq. 3: How does the transition rate depend upon membrane potential? More specifically, to what extent is the low field limit (Eq. 16) applicable, and what are the values of the effective dipole moment differences (M_{jk} in Eq. 16)? When the low field limit fails, will the next approximation (Eq. 19) be sufficiently accurate, or is the actual case far from the low field situation (for example, Eq. 20 for the simplest case)? I will indicate briefly the approach my laboratory has taken, or is taking, to each of these questions.

Because the diagram for excitable membrane channels is not known, experimental investigation of transition rates between various conformations is difficult; measured relaxations in general reflect multiple transition rates and a transition that dominates at one voltage need not be appreciable at other voltages. In view of the difficulties with studies of this question on electrically excitable membranes, we have turned to the study of voltage effects on gating in a model system. Specifically, we have investigated voltage effects on the closing rate of channels activated by acetylcholine at the frog neuromuscular junction.

In our original investigations (Magleby and Stevens, 1972; Anderson and Stevens, 1973; Dionne and Stevens, 1975) we found that the logarithm of the closing rate constant depends linearily on membrane potential. That is, the transition rate from the open to the closed conformation has a voltage dependence described accurately by Eq. 16. As Magleby and Stevens (1972) pointed out, the logarithm of the rate constant should, for large enough voltages, exhibit a dependence on V^2 (see Eq. 19), but they were unable to detect the presence of this effect. The low field approximation thus appears adequate over the entire range of voltages realizable in these voltage clamp experiments (-150 to $+50$ mv). The normal component of the dipole moment change between the open conformation and the transition state (M in equation 16) is about 50 D.

The experiments discussed above were done on *Rana pipiens*. More recently, E. Neher, J. H. Steinbach, and I (unpublished observations) have done similar experiments on neuromuscular junctions from *Rana temporaria* and found that the logarithm of closing rate varies nonlinearly with voltage. In this case, the transition rate constant has the form given by Eq. 19. Our results could be accounted for if the open state of the channel has a dissociable charge buried partway within the membrane that has a somewhat different binding constant in the two species of frog.

The conclusion from our observations is that, at least for the acetylcholine-activated channel, the low field approximation gives an adequate characterization of the transition rate constant over a wide voltage range in one instance, and over a somewhat more restricted range in another. If our experience with the acetylcholine-activated channel at the neuromuscular junction can be extrapolated to the nerve membrane, we would answer question (*c*) by tentatively supposing transition rates to be described by Eq. 16 for at least a restricted voltage range, but would anticipate that an equation of the form of Eq. 19 would be necessary in at least some cases.

To discover the conductance associated with each conformation of a channel without knowing what or how many conformations the channel can assume is, of course, difficult. Nevertheless, some progress can be made on this question by using fluctuation analysis. The basic idea is to discover whether the single channel conductance (estimated by fluctuation analysis: see e.g., Neher and Stevens, 1977) has the same value for all membrane potentials, or varies (say) in parallel with the overall membrane conductance. If excitable membrane channels, like acetylcholine-activated channels (Neher and Sakmann, 1976), have only two conductance states, open and closed, the single channel conductance should—except for single channel rectification that can be taken into account by measuring the instantaneous voltage-current relation—have the same value for all membrane potentials. If, on the other hand, channels have lower conductances in some conformations and higher in others, the single channel conductance should reflect the higher and lower values at voltages that tend to favor particular conformations. Unfortunately, this test can be less definitive than one would like. If the measured single channel conductance is not independent of voltage, this implies that something more complicated than identical, independent open-shut channels is present. A single channel conductance that does not vary with membrane potential is most simply explained by an open-shut channel mechanism, but specific example of channels with multiple conductances that give the same result can easily be constructed.

Preliminary answers to question (*b*) have been obtained by Begenisich and Stevens (1975) for potassium channels, and by Sigworth (1977) for sodium channels in frog node of Ranvier. Both studies found that single channel conductance is independent of membrane potential with a value of about 4 pS for potassium channels and value about twice that for sodium channels. A tentative answer to question *b*, then, is that all conformations have one of two conductances depending on whether the channel is open or closed. If this simplest conclusion is incorrect, then at least very definite constraints are placed upon the possible distribution of conductance among the channel conformations. Specifically, the system must give an approximately constant value for the quantity $\sigma^2/(g(1 - f))$ where σ^2 is the varience of conductance fluctuations, g is the mean conductance, and f is the ratio of g to the maximum obtainable conductance.

The most difficult problem, within the context of the theory presented here, is to answer question *a*, that is to determine the diagram for a channel. The standard approach, originally adopted by Hodgkin and Huxley (1952), is to infer a kinetic mechanism from the average behavior of a population of membrane channels studied under voltage clamp. This approach is still the best source of information about the channel diagram, but unfortunately cannot place enough constraints on possible mechanisms to permit a decision between various plausible alternatives. I have given a specific example of two physicially different mechanisms that give identical predictions about the results of any conceivable measurements of mean ionic currents, (Stevens, 1972) and it is not difficult to construct others. Further, because of inprecision in measurements, an even larger number of diagrams are, in practice, indistinguishable.

Although different diagrams may make indistinguishable predictions about mean currents flowing through a population of channels, two classes of experiments can, in general, permit discriminations between mechanisms. The first approach is to measure gating currents, that is, the currents associated with the change of effective dipole moment (M in Eq. 16, for example) associated with the movement of the channel from one to another conformation (see Armstrong, 1975). If the voltage dependence of transition rates is known, then a diagram permits predictions to be made about gating currents. The value of this approach is that distinct diagrams that predict about the gating currents. A version of this approach has recently been pursued by Armstrong and Bezanilla (1977).

A second approach is to study the spectrum of conductance fluctuations that arise from the random opening and closing of channels. This spectrum can, by standard procedures, be predicted from Eq. 2 and a knowledge of the conductances associated with each conformation. In general, different diagrams give different spectra even when the predicted mean currents are indistinguishable. A specific example of how this approach might be used has been given earlier (Stevens, 1972) and Ruff (1977) has recently employed the method to distinguish between diagrams associated with different possible local anesthetic actions at the postsynaptic membrane channels. My laboratory is currently pursuing this program in an attempt to discover the diagram for channels in the frog node of Ranvier membrane; that is, we are trying to get an answer for question *a*.

If the theoretical framework presented here proves to be appropriate for understanding nerve excitability, the study of gating currents and fluctuations should be the key for discovering channel diagrams, and could then eventually lead to a complete theory for electrical excitability.

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