# SENSITIVITY OF MEMBRANES TO THEIR ENVIRONMENT

## Role of Stochastic Processes

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ABSTRACT Ionic flow through biomembranes often exhibits a sensitivity to the environment, which is difficult to explain by classical theory, that usually assumes that the free energy available to change the membrane permeability results from the environmental change acting directly on the permeability control mechanism. This implies, for example, that a change $\Delta V$ in the *trans*-membrane potential can produce a maximum free energy change, $\Delta V \cdot q$, on a gate (control mechanism) carrying a charge $q$. The analysis presented here shows that when stochastic fluctuations are considered, under suitable conditions (gate cycle times rapid compared with the field relaxation time within a channel), the change in free energy is limited, not by the magnitude of the stimulus, but by the electrochemical potential difference across the membrane, which may be very much greater. Conformational channel gates probably relax more slowly than the field within the channel; this would preclude appreciable direct amplification of the stimulus. It is shown, however, that the effect of impermeable cations such as $Ca^{++}$ is to restore the amplification of the stimulus through its interaction with the electric field. The analysis predicts that the effect of $Ca^{++}$ should be primarily to affect the number of channels that are open, while only slightly affecting the conductivity of an open channel.

### INTRODUCTION

Biological membranes usually exhibit a high sensitivity to their environment so that relatively small changes may result in large changes in ionic permeability. The change in the environment may be, for example, mechanical, thermal, chemical, or electrical. The cochlear hair cells are an example of a highly sensitive mechanoreceptor (1), while the firing rate of certain hypothalamic neurones is critically dependent on the temperature (2). Examples of chemical changes are the action of various transmitter substances at synapses and effector organs, for example, the effects of various hormonal agents such as aldosterone on renal function (3), as well as the effect of ionic concentrations.

An example of high sensitivity to electric fields is found in certain electrically sensitive fish (4), which can respond to fields as low as 0.1 mV/cm. A less extreme example, but one in which the sensitivity has up to the present not been adequately explained, is the axon. A great deal of quantitative experimental work has been done on the axonal membrane, with much of the information expressed by the well-known Hodgkin-Huxley equations (5).

In this paper I will largely confine my discussion to electrically excitable membranes and will use the axon as an example. The change in the ionic permeability of the membrane may be thought of as being controlled by "gates" carrying an electric charge. Although several specific gating mechanisms will be considered, the term gate is used in a general sense to mean any mechanism by which the flow of ions through the membrane is controlled.

For simplicity we will consider gates, that are either fully open (which will be termed the $p$ state), or fully closed (the $q$ state); i.e., "binary gates." Then in the steady state we may write

$$\overline{P}/\overline{Q} = k_e. \tag{1}$$

$\overline{P}$ is the fraction of all the gates that are open, and $\overline{Q}$ the fraction closed; $\overline{P} + \overline{Q} = 1$. $k_e$ is the (quasi) equilibrium constant for the $p$-$q$ reaction. Each gate is assumed to be associated with an ion-permeant channel, so that $\overline{P}$ and $\overline{Q}$ are then the fraction of all the channels open and closed. Because the gates will open and close stochastically, $\overline{P}$ and $\overline{Q}$ are also fractions of the time a gate is open or closed. For simplicity, all channels are assumed to be identical.

We now consider that each gate carries a charge $zq$; $z$ is the number of electron charges $q$; $z$ is positive if the gate charge is positive. If now an electric field is applied across the membrane, so that when moving from closed to open the gate moves through a potential difference $\Delta V$, the free energy required to open the gate will be increased by an amount $\Delta G = zq\Delta V$. This change in free energy for the $p$-$q$ reaction will, by Boltzmann's relation, shift the then steady state by a factor $e^{-zq\Delta V/kT}$, so that we now have

$$\overline{P}/\overline{Q} = k_e e^{-zq\Delta V/kT}. \tag{2}$$

Since $kT/q \sim 25$ mV, it is evident that, if gates are singly charged, the maximum that the conductivity could

be increased by a stimulus of 25 mV acting directly on the gate would be a factor of e; i.e., ~2.7.

Hodgkin and Huxley found the $Na^+$ conductance to increase at six or more times this rate for voltage changes near the resting potential. It has been proposed that the gating mechanism may involve six or more electron charges that act as a unit. This is a possibility, although no molecular structure has been identified with such characteristics. For the higher sensitivities as must exist in the electrically sensitive fish, such an explanation is proportionally more difficult (6).

In considering the implications of Eq. 2, it has previously been assumed that the value of $\Delta V$ (the voltage change acting on the gating mechanism) is limited to the change in the voltage across the membrane, i.e., the voltage of the stimulus. In an equilibrium system this would be true. However, excitable membranes, in common with most if not all biomembranes, exist in systems far from equilibrium. Thus, in an axon in its resting state, while $K^+$ ions are close to electrochemical equilibrium, $Na^+$ ions have an almost reciprocal concentration gradient and are thus far from electrochemical equilibrium of the order of 150 mV, which is equivalent to a free energy difference across the membrane of ~6 kT. It is this energy that can be mobilized by a much smaller stimulus as a result of the stochastic opening-and-closing of the gates. This can then result in an apparent amplification of the power of the stimulus. This amplification can be very high under suitable conditions (7).

## ELECTRIC FIELD THROUGH A MEMBRANE

Fundamental to understanding the origins of the sensitivity of membranes is an understanding of the nature of the electric field through the membrane and how it will be affected by local changes in membrane conductivity, i.e., by diffusion barriers. That the local electric field will be so affected has been recognized since Planck's original work on ionic diffusion. Although the Goldman constant field equation (8) has been a convenient approximation, it masks this essential fact and its important ramifications.

An exact calculation of the electric field in a membrane channel would be very difficult even if we knew the actual structure of a channel; and even calculations based on simplifying assumptions are complex. But because we have no real knowledge of the channel's structure, we need do no more than show the general nature of the change in the field, which must take place with the opening and closing of a gate.

We first, in Fig. 1, consider a very simple limiting case: a neutral (uncharged) uniform conducting channel (*a*) between two electrolyte baths, held at a potential difference of 100 mV. The channel will act substantially as a resistor (*b*). When the gate (of any, unspecified nature) is open, equivalent to having the switch at *b* closed, the potential will fall uniformly across the channel (constant field). The potential difference across the gating region will thus be low. When the gate is closed, on the other hand (switch open), all the voltage drop appears across the gate.

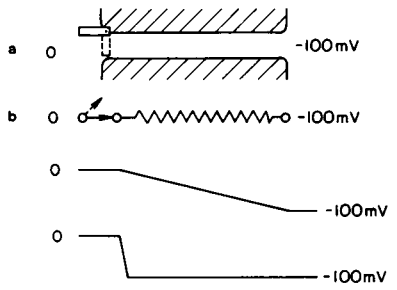


FIGURE 1 (*a*) Schematic representation of a gated ionic channel. (*b*) Electrical circuit analog of the channel. Gate in its open (——) position at *a* corresponds to the closed switch position at *b*; the voltage drop occurs in the resistor, i.e., along the channel. When the gate is closed (---), corresponding to the switch being open, all the voltage drop is across the switch or gate.

A more realistic (but still highly simplified) channel model is shown in Fig. 2. This channel has negative fixed charges uniformly distributed along its walls. For simplicity, we assume that the ionic concentrations in the two baths are equal, but that a potential difference of 100 mV is maintained between them. We also assume that the channel is impermeable to anions. Under these conditions, when the gate is open, as in Fig. 2 *a*, the cation distribution will just balance the negative charge along the walls, and the electric field through the channel will be constant.

We now consider what will happen if the channel gate is closed, as shown in Fig. 2 *b* where the channel is blocked by a divalent cation, e.g., $Ca^{++}$. Because there will now be no ionic flow, at every point along the channel the cations

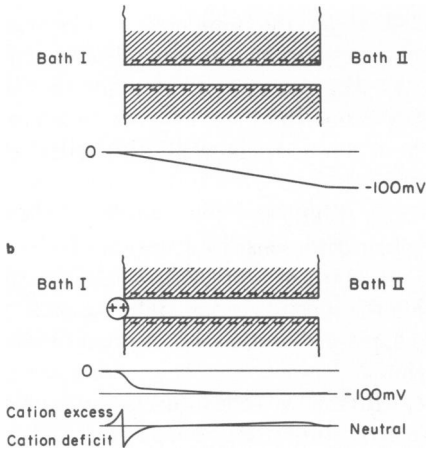


FIGURE 2 Cation selective channel between baths differing in potential by 100 mV, showing how the voltage distribution changes as a result of a change in ionic distribution through the channel when the gate closes (here shown as by the adsorption of a divalent cation). *a*. Channel open, electroneutrality through the channel, and a constant field. *b*. Channel closed, showing cation excess and deficit resulting in the large change in electric field at the interface. Baths assumed to be of equal ionic strength and equal to the fixed charge density of the channel.

must be at equilibrium. This means that any concentration gradient that may exist anywhere in the channel must be exactly balanced by the local electric field; that is

$$kT\frac{dC}{dx} = EFC. \quad (3)$$

$F$ is the value of the Faraday constant, and $C$ is the local (univalent) cation concentration. Where there is no concentration gradient, the electric field must evidently be zero; but elsewhere there must be an electric field that will integrate across the channel to equal the potential difference maintained between the two baths. The change in the electric field must be accompanied by a departure from electroneutrality, because by Poisson's equation

$$\nabla E = \rho/\epsilon. \quad (4)$$

$\rho$ is the net positive charge density, and $\epsilon$ is the permativity of the medium. Thus the change in electric field to that corresponding to the potential contour shown in Fig. 2 *b* will only be complete when the ions have redistributed along the channel and within the double layers to their new steady state distribution, as shown by the lower curve in Fig. 2 *b*.

It is apparent that if the gate were to close when the electric field is as shown in Fig. 2 *a*, there would be little potential difference at the interface and thus only a small electrostatic energy to hold the gate closed. If, however, the gate were to remain closed for a long period, the field would take on its new form corresponding to a closed gate, and substantially the full 100 mV could be effective in holding the gate closed, e.g., in binding the $Ca^{++}$ ion.

The increased binding energy would then increase the fraction of time the gate remains closed; that is, it would decrease the $\overline{P}/\overline{Q}$ ratio, Eq. 2. Thus if a gate remains closed for a long period, the probability of it remaining closed is increased; the opposite occurs if the gate remains open for a long time. It is thus seen in this example that the electric field changes to favor the current state of the gate; this is essential for enhanced sensitivity.[1] If the effect on the gate were the contrary, e.g., for gating by an adsorbed anion, the result would be diminished sensitivity of the channel to adsorption of such an ion.

The actual nature of the ionic channels in membranes remains largely unknown, but surely does not closely resemble the above example. However, calculations have been made on the change of voltage distribution in a wide variety of other more complex and possibly more realistic channel models (9–11); all show a qualitatively similar change in the voltage across the gating region, but the magnitude of the effect depends, as would be expected, on the details of the model.

The actual gating mechanism of the channel is also unknown. $Ca^{++}$ has been used in the above example because of the demonstrated action of $Ca^{++}$ on ionic conductivity in axon (12), an effect that may be simply and quantitatively explained by such gating (9, 14, 15). It is almost certain, however, that conformational changes play an important role. Such changes could involve simple deflections of ionized side chains (15)[2] or more complex structural rearrangement in membrane proteins.

Two time factors will be of significance in the theory. These are the mean open and close times of the gates, and the relaxation times of the voltages across the gates with the opening and closing of the gates. It is the ratio of these time factors that are of importance: the sensitivity increases with an increase in the ratio of the relaxation time of the voltage to the dwell times of the gates.

Considering first $Ca^{++}$ ion adsorption as a gating process, collision theory indicates that the gating period will probably be $<10^{-7}$ s. It is much more difficult to estimate even an order of magnitude for the conformational gating times. Simple electrostatic deflection of a side chain should be quite fast, probably $<10^{-6}$ s, but the time of conformational changes of large protein molecules might be on the order of milliseconds. There is now evidence that some gating processes have a mean open time of several milliseconds, as shown by single-channel recordings (16–19), but in membranes responding more slowly than do the $Na^{+}$ gates in axon. The relaxation times of the gates are even more difficult to estimate theoretically, given the lack of knowledge of the channel structure. There are, however, some data that may be pertinent.

Benz and Conti (20) measured the relaxation of membrane voltage of squid axon in response to a brief charge pulse. They found one component of the voltage to have a relaxation time constant of 100 to 200 $\mu$s. They attributed this component to be due to movement of gating particles within the membrane. Similar results were obtained by Meves (21) for the polarized squid axon, with the time constant less by a factor of $\sim$10 in the depolarizing sense. According to the theory presented here, the "gating particle" movement would be the ionic displacement within the channels, giving rise to a voltage shift, and thus resulting in the opening and closing of the channel gates. This is consistent with the present interpretation (22) that gating currents are due to charge motion within the membrane, resulting in a change in the interface potentials. However, the gating currents result from an externally applied electric field across the membrane, and thus should be more rapid than the ionic relaxation occurring as a result of the spontaneous opening and closing of the channel gates.

How these two time factors can result in high sensitivity of the membrane may be seen graphically from the curves of relaxation of the gate potential, as illustrated in Fig. 3.

[1]When the effect if stochastic fluctuation of the gating process is neglected, the effect appears to predict two stable states (9).

[2]This paper did not adequately consider the effect of the Boltzmann distribution of gate states. An investigation of this factor resulted in the work presented here.

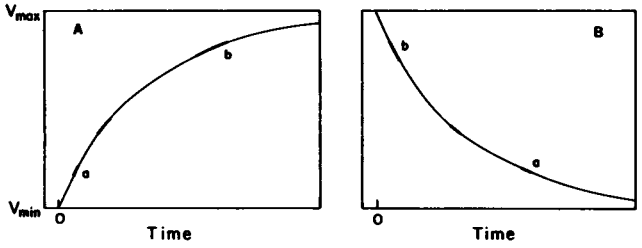


FIGURE 3 Graphical explanation of the origin of high membrane sensitivity. (*A*) Rise in potential across the gate when it closes. Because the closed dwell time increases exponentially with the potential across the gate, the mean dwell time (shown by the weighted segments) at *b* is much longer than at *a*, but the rate of rise of $\Delta V$ is lower. (*B*) Since the open time is assumed to be independent of potential across the gate, the open times at *a* and *b* are the same, but because of the steeper slope at *b*, the mean decrease in potential is higher at *b* than at *a*. Under the conditions illustrated in the figure, the mean up- and down-swings of potential at both points *a* are equal, as are the swings at both points *b*. The result is the possibility of two steady state distributions: at *a* and *b*.

Here at *A* the increase in the potential at the interface is plotted, as would occur if the gate were held closed after a long open period; and at *B* the opposite is plotted, the gate being held open after having been closed a long time.

The curves are plotted assuming first-order (exponential) relaxations with equal time constants. We as an example consider a $Ca^{++}$ ion as the gate. The mean time the ion will remain adsorbed, and thus the gate remain closed, is

$$t_q = k_1 \exp(2e\Delta V/kT), \tag{5a}$$

while the open time is assumed independent of the potential[3]

$$t_p = k_2. \tag{5b}$$

$k_1$ and $k_2$ are constants independent of $\Delta V$; $k_e$ in Eq. 2 is equal to $k_1/k_2$.

In Fig. 3 *A*, the the mean change in $\Delta V$ of a channel residing at point *a* with the gate closed is shown by the heavy line segment; the closed dwell time, $t_q$, is short, since $\Delta V$ is small. Thus, although the curve rises steeply, the (mean) change in $\Delta V$ is small.

We compare this now with the corresponding point *a* in Fig. 3 *B*. Here, the (constant) open dwell time, $t_p$, is longer than $t_q$ was, but the slope of the curve is much lower. The result is that the mean change in $\Delta V$ at *a* when the gate is open (Fig. 3 *B*), is equal, and opposite to the corresponding point in Fig. 3; this then corresponds to a possible steady state of the channel.

A similar analysis applies to the two heavy line segments marked *b*, but here $\Delta V$ at the first point rises more slowly, but for a longer time; $t_q$ is longer at the higher value of $\Delta V$. At the corresponding point where the gate is open, $\Delta V$ falls more rapidly, again for a time $t_p$; the result again is that the two mean changes in $\Delta V$ are equal and opposite. This then also corresponds to a possible steady state of the channel.

Thus with these exponential relaxation functions, there are two possible steady state points, *a* and *b*; how the actual steady state will be distributed between the two values will be discussed below. With other relaxation functions there may be but one point where the down shifts are equal. But since $t_q$ is sensitive to $\Delta V$, this point (the steady state value of $\Delta V$) may be similarly sensitive to the maximum value of $\Delta V$, that is, to the potential across the membrane, or to other factors that may cause changes in $k_1$ or $k_2$, e.g., ionic concentrations.

A channel at steady state residing, for example, at *a* in Fig. 3, will be stochastically opening and closing, wandering up and down in voltage across the gate in a random walk, but remaining near *a*, as shown in Fig. 4 *a*, with the corresponding low value of $\Delta V$, and a high value of $\overline{P}/\overline{Q}$ (Eq. 2). The channel is open most of the time, and most of the channels in the membrane will be open at any instant.

If now a parameter of the membrane is changed; e.g., the voltage across it is raised, favoring the state *b*, the gates will remain closed a little longer, biasing the random walk towards a higher voltage across the gate, as shown at *b* in Fig. 4. The fluctuations will gradually urge the gates towards predominantly the *b* location, representing a higher value of $\Delta V$, and a lower $\overline{P}/\overline{Q}$ ratio; most of the channels will now be closed. After having given this intuitive discussion, the problem will now be treated analytically.

The random nature of the process we are investigating is the result of at least two stochastic processes: first, the randomness of the gate opening and closing; and second, the inherent fluctuation noise in the voltage across the gate. This latter source of randomness will include that due to the Brownian motion of the ions, which conducts current through the membrane as predicted for any conductor by Einstein (24), another example of the familiar Johnson (Nyquist) noise; there may be other sources also contributing to this noise, such as ion shot noise (25).

The system will first be analyzed including both sources of stochastic fluctuations. Two special cases will then be treated. The first is that in which the gate dwell times are so short that their stochastic fluctuation has a negligible effect, but does include the fluctuation noise in the voltage. This case should be applicable to gating by adsorbed cations having a short dwell time; e.g., $Ca^{++}$. The second case is one where the gates operate more slowly, but the voltage noise is negligible. It will be found that the effects of both sources of noise are essentially the same: the broadening of the voltage sensitivity of the response.

A system in which both types of gating operate together

[3]This assumption is made for simplicity; it does not affect the result of the analysis. The conditions under which this would be a good approximation for gating by $Ca^{++}$ are discussed in reference 13. Patch clamp experiments have shown $t_p$ to be almost independent of voltage in mammalian $Na^+$ channels (23); whether this is equally the case in other channels remains to be determined.

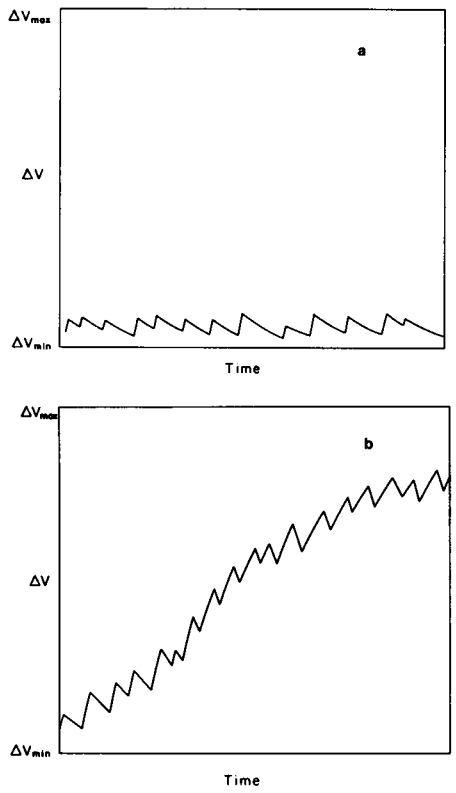


FIGURE 4 Quasi-random walk of potential across gate. (*a*) Channel residing at a steady state point of high $\Delta V$ across the gate, such as point *b* in Fig. 3. Channel remains largely in the closed (*q*) state. (*b*) Channel conditions changed slightly, to favor the open (*p*) state: the channel state walks towards a lower value of $\Delta V$, and a predominantly open (*p*) state.

is then treated. This should most nearly represent the normal excitable membrane in which relatively slow, presumably conformational, gating, and $Ca^{++}$ ions in the external medium join in affecting membrane conductance. It will be shown that the two gating means do not merely add their respective effects, but that the $Ca^{++}$ can "catalyze" the conformational gate by its effect on the electric field.

The mathematical analysis of a system involves theoretical considerations of a certain degree of abstraction as well as a mathematics that tends to become somewhat complicated. Readers who are more interested in the results of the theory than in following its mathematical details may wish to skip some of the following sections, including parts or all of the sections entitled Analysis of Stochastic Gating, Analysis of the Equations of State, Sensitivity for Very Fast Gating, and Analysis of $Ca^{++}$ Effect on Confirmational Gating.

## ANALYSIS OF STOCHASTIC GATING

In our treatment of the problem, in Eq. 5a we define

$$k_0 = k_1 \exp(ze\Delta V_{max}/kT). \tag{6}$$

$z$ is the number of electron charges carried by the gate, and $\Delta V_{max}$ is the value $\Delta V$ will have when the gate has been closed a long time.

In practice, and as already discussed, the gates will be opening and closing stochastically, so that $\Delta V$ will have a spectrum of values $<\Delta V_{max}$ for the various channels of the membrane. The free energy holding a channel's gate closed will then be, in units of $kT$, less than its maximum value by an amount $\gamma = ze(\Delta V_{max} - \Delta V)/kT$. In this notation, we rewrite Eq. 5

$$t_q = k_0 e^{-\gamma}, \tag{7a}$$

while Eq. 5b remains unchanged, since we have assumed all the variable electrical free energy goes towards holding the gate closed:

$$t_p = k_2. \tag{7b}$$

If a gate were to be held open for a long time, then, as shown in Fig. 3, $\Delta V$ would assume its lowest value, $\Delta V_{min}$, and $\gamma$ would take on its highest value $\Gamma = ze(V_{max} - V_{min})/kT$. Thus $\gamma$ will vary between 0 and $\Gamma$; it will relax towards a mean value of $\Gamma$ when the channel is in the *p* (open) state, and towards a mean value of 0 when in the *q* (closed) state. Neglecting for the moment the effect of voltage noise, we may thus write

$$d\gamma/dt = f_p(\Gamma - \gamma) \tag{8a}$$

when in the *p* state, and

$$d\gamma/dt = -f_q(\lambda) \tag{8b}$$

when in the *q* state. The relaxation functions, $f_p$ and $f_q$, are monotonically increasing functions of their respective arguments, and are evidently 0 when the argument is 0, but may otherwise take any functional form. The simplest would be first-order kinetics

$$d\gamma/dt = \alpha_p(\Gamma - \gamma); \tag{9a}$$

$$d\gamma/dt = -\alpha_q\gamma, \tag{9b}$$

respectively.

We now consider a system consisting of a large number of such members. Let $P = P(\gamma)$ be the density of members of the system in state *p* having an energy shift $\gamma$. That is, $Pd\gamma$ is the fraction of the total number of members that are in the *p* state, and have an energy shift from $\gamma$ to $\gamma + d\gamma$, and similarly for *q*. Then

$$\int_{-\infty}^{\infty} (P + Q)d\gamma = 1. \tag{10}$$

Integration is from $-\infty$ to $+\infty$ rather than from 0 to $\Gamma$, because the fluctuation noise in $\Delta V$ allows $\gamma$ to vary beyond its nominal limits.

The system is thus characterized by two parameters at a given instant: the value of $\gamma$ for each member, and its present state, either *p* or *q*. We will calculate the rate of

change of density of members at each value of the $\gamma$ coordinate of the system; that is, the probability of a member having an energy shift $\gamma$; and, at each value of $\gamma$, the distribution of members between their $p$ and $q$ states. This calculation will be made by considering the "flow" of members in each state along their $\gamma$ coordinate, as well as their cross flow from one state to the other, i.e., the $p$-$q$ transition. This will give a partial differential equation describing the time course of the state of the system following any perturbation applied to the system; by setting the time derivative equal to 0, the steady state configuration of the system will be obtained.

Considering the members of the system in state $p$, at an energy shift $\gamma$, these members will flow towards $\gamma = \Gamma$ at a rate $J_{p1} = P \cdot f_p(\Gamma - \gamma)$ due to the driving force given by Eq. 9a, analagous to the Coulombic driving force on ions in the Nernst-Planck equation.

In addition, the probability density gradient of members in the $p$ state will result in a diffusive flow due to the stochastic fluctuation in $\gamma$:

$$J_{p2} = -D_p dP/d\gamma.$$

This is directly analagous to the Fick's law diffusion term in the Nernst-Planck equation, which results from the Brownian motion of molecules in solution; here, the Brownian motion is in the electric charge.

Adding these two flows, we have for the total flow of probability density for members in the $p$ state

$$J_p = P \cdot f_p(\Gamma - \gamma) - D_p dP/d\gamma. \quad (11a)$$

For members in the $q$ state, we have the corresponding equation

$$J_q = -Q \cdot f_q(\gamma) - D_q dQ/d\gamma. \quad (11b)$$

Eqs. 11a and b are forms of the Fokker-Planck equation. The Einstein coefficients, $D_p$ and $D_q$ represent the randomness of the $\gamma$-field; an estimation of their values is given in Appendix A.

The flows will not be constant over $\gamma$, so that there will be a change in the local density of elements at coordinate $\gamma$ due to flow. Applying the continuity equation gives

$$(\partial P/\partial t)_j = -\partial J_p/\partial\gamma = -P \cdot \partial f_p/\partial\gamma - f_p \cdot \partial P/\partial\gamma + D_p\partial^2 P/\partial\gamma^2 \quad (12a)$$

$$(\partial Q/\partial t)_j = -\partial J_q/\partial\gamma = Q \cdot \partial f_q/\partial\gamma + f_q \cdot \partial Q/\partial\gamma + D_q\partial^2 Q/\partial\gamma^2. \quad (12b)$$

In addition, there will be a change in density of elements in state $p$ at coordinate $\gamma$, due to the transition from state $p$ to $q$ and vice versa. The rate constants for these changes in state density are obtained from Eqs. 7a and b, and are the reciprocal of the respective dwell times: $k_p = 1/k_2$ for the $p$ state, and $k_q e^\gamma$ for the $q$ state, with $k_q = 1/k_0$. These then give the rates of change of state densities due to the $p$-$q$ reaction

$$(\partial P/\partial t)_r = k_q e^\gamma Q - k_p P \quad (13a)$$

$$(\partial Q/\partial t)_r = k_p P - k_q e^\gamma Q. \quad (13b)$$

The total rate of change of state density coordinate is then given by adding Eqs. 12 and 13

$$\partial P/\partial t = -(k_p + \partial f_p/\partial\gamma)P + k_q e^\gamma Q - f_p\partial P/\partial\gamma + D_p\partial^2 P/\partial\gamma^2 \quad (14a)$$

$$\partial Q/\partial t = k_p P - (k_q e^\gamma - \partial f_q/\partial\gamma)Q + f_q\partial Q/\partial\gamma + D_q\partial^2 Q/\partial\gamma^2. \quad (14b)$$

To find the steady state, we set the time derivatives equal to 0, giving

$$dP/d\gamma = [-(k_p + df_p/d\gamma)P + k_q e^\gamma Q + D_p d^2 P/d\gamma^2]/f_p \quad (15a)$$

$$dQ/d\gamma = [-k_p P + (k_q e^\gamma - df_q/d\gamma)Q + D_q d^2 Q/d\gamma^2]/f_q. \quad (15b)$$

Integration of Eqs. 15a and b over the region of $\gamma = -\doteq$ to $\gamma = \doteq$ and normalizing by application of Eq. 10 then gives the fraction $\overline{P}$ of all the members that are in the $p$ (open) state: this is the membrane conductivity, as a fraction of all channels in the conducting state. Evidently $\overline{Q} = 1 - \overline{P}$.

If the relaxation functions, $f_p$ and $f_q$, are first order, Eqs. 14a and b become

$$\partial P/\partial t = -(k_p - \alpha_p)P + k_q e^\gamma Q - \alpha_p(\Gamma - \gamma)\partial P/\partial\gamma + D_p\partial^2 P/\partial\gamma^2 \quad (16a)$$

$$\partial Q/\partial t = k_p P - (k_q e^\gamma - \alpha_q)Q + \alpha_q\gamma\partial Q/\partial\gamma + D_q\partial^2 Q/\partial\gamma^2, \quad (16b)$$

and for the steady state Eqs. 15a and b become

$$dP/d\gamma = [-(k_p - \alpha_p)P + k_q e^\gamma Q + D_p d^2 P/d\gamma^2]/\alpha_p(\Gamma - \gamma) \quad (17a)$$

$$dQ/d\gamma = [-(k_p P + (k_q e^\gamma - \alpha_q)Q + D_q d^2 Q/d\gamma^2]/\alpha_q\gamma. \quad (17b)$$

## ANALYSIS OF THE EQUATIONS OF STATE

Before solving Eqs. 17a and b numerically, it will be instructive to examine the behavior of the equations for the limiting case of vanishingly small relaxation constants, $\alpha_p$ and $\alpha_q$ that is, when the relaxation of the electric field is very slow compared with the gating frequency. We will at the same time take $D_p$ and $D_q$ to be very small (little randomness in the electric field), so that the second derivative terms may be omitted. In the limit, Eqs. 15a and

b then become

$$dP/d\gamma = (-k_p P + k_q e^{\gamma} Q)/\alpha_p(\Gamma - \gamma) \quad (18a)$$

$$dQ/d\gamma = (-k_p P + k_q e^{\gamma} Q)/\alpha_q \gamma. \quad (18b)$$

Because the values of $\alpha_p$ and $\alpha_q$ and are vanishingly small, everywhere either $P$ and $Q$ must be extremely small or

$$k_p P = k_q e^{\gamma} Q. \quad (19)$$

That is

$$P/Q = k_q e^{\gamma}/k_p. \quad (20)$$

Eq. 20 will be satisfied at the maxima and minima of $P$ and $Q$; in the limit $P$ and $Q$ will be extremely small except at the maxima, where they will become delta functions.

For stationarity, the flow of channel densities, $J_p$ and $J_q$, in the two senses (towards $\gamma = \Gamma$ and $\gamma = 0$) must be equal and opposite; i.e., $J_p = -J_q$. Then by Eqs. 11a and b (but recalling that the second derivative terms are omitted here, we have the additional condition

$$P/Q = f_q/f_p, \quad (21)$$

which is, for first-order relaxation

$$P/Q = \alpha_q \gamma/\alpha_p(\Gamma - \gamma). \quad (22)$$

The simultaneous solution of Eqs. 20 and 22 then gives the positions of the maxima and minima of $P$ and $Q$.

These positions may be found by plotting $P/Q$ vs. $\gamma$ for Eqs. 20 and 22. This is shown in Fig. 5 by the solid line curves. In semilogarithmic coordinates Eq. 20 becomes a straight line, and Eq. 22 skewed. The figure is plotted for $\alpha_p = \alpha_q$, $\Gamma = 6$, and $k_q/k_p = e^{-\gamma/2}$. Here there are three points of intersection: two maxima, with a minimum between. With these symmetrical conditions, the total population at the two maxima would be equal, with reciprocal population ratios: $P/Q = 0.075$ at the lower intersection, and $P/Q = 13.2$ at the upper. The value $\Gamma = 6$ indicates the total electrochemical energy across the membrane that is available to affect the channel gate; this could, for example, be a 150-mV electrochemical potential difference acting on a singly-charged gate, or 75 mV acting on a doubly-charged gate. A lower value of $\Gamma$ would result in a lesser change in the $P/Q$ ratios, and the opposite for a higher value.

This distribution is purely theoretical for the limiting case, because any finite disturbance of any parameter would cause the total population to shift to either the lower or upper intersection.

In this example, the high limiting sensitivity is due to the existence of two maxima; that is, the fact that the two curves representing Eqs. 20 and 22 in Fig. 5 have three points of intersection. With other relaxation functions, which could, for example, result in the dashed curve of Fig. 5, there could be only a single point of intersection, and thus the channel population would be distributed about a single value of $\gamma$. While the sharpness of the distribution would depend, as before, on the ratio of the dwell to field relaxation times, the steepness of change in $P/Q$ with membrane parameters (such as $k_q$) would depend upon the form of the equation representing the dashed curve of Fig. 5; i.e.,

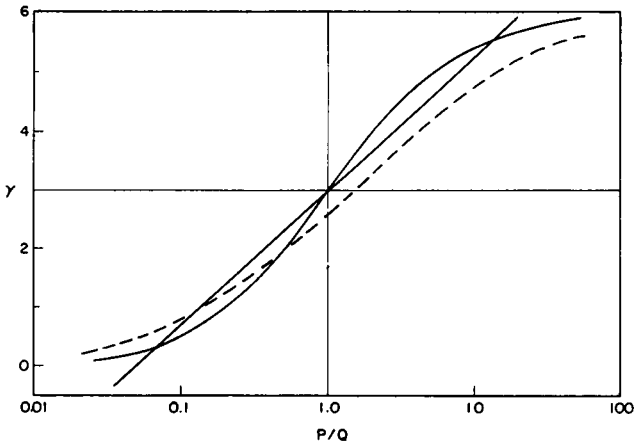


FIGURE 5 Graph showing limiting solutions of Eq. 17, or Eq. 36. Straight line is a plot of $k_e e^{\gamma}$, and curved lines are plots of $f_q/f_p$: (solid skewed) curve is for first-order relaxation of the electric field, which results in three points of intersection; the outer two are possible maxima of channel distribution function. Dashed line (---) represents a different possible $f_q/f_p$ function, which would result in only one point of intersection, and thus only one maximum in the distribution. The abscissa is also $f_q/f_p$.

$$P/Q = f_q(\gamma)/f_p(\Gamma - \gamma). \quad (23)$$

If this curve should closely parallel the straight line representing Eq. 20, the sensitivity would be high; as the curves diverge in slope, the sensitivity becomes less, but always greater than would be predicted by classical theory. The relaxation curves must depart rather drastically from exponential to avoid having the three points of intersection, and thus a bimodal distribution. Since recordings of asymmetry currents, which should be of a form similar to the field relaxation, appear to be nearly exponential (21), the calculations to be presented will, for definiteness, assume first-order relaxations. Some calculations have, however, been made with relaxation curves resulting in only a single intersection; the results did not differ significantly, with parameters corresponding to a realistic degree of randomness.

## EFFECT OF MEMBRANE PARAMETERS

### Influence of $f_q/f_p$ Ratio

If the relaxation time constants, $\alpha_q$ and $\alpha_p$, as Eq. 22 shows, are not equal, the skew curve of Fig. 5, will move to the left if $\alpha_q < \alpha_p$, and vice versa. If at the same time $k_1/k_p$ is

changed by the same factor as $\alpha_p/\alpha_p$ (or, more generally, as $f_q/f_p$), the curves in Fig. 5 will remain in the same relationship to one another, but will be moved along the $P/Q$ axis.

Thus, for example, if the relaxation time of the field with the gate closed is ten times its value with the gate open, as suggested by the results of Meves (21), the $P/Q$ ratios would, in the limiting case illustrated by the curves of Fig. 5, become 0.0075 for the lower (resting) and 1.32 for the upper (conducting) state.

Remembering that $\overline{P} + \overline{Q} = 1$, the value of $\overline{P}$ in the conducting state is then 0.57, while in the resting state it is 0.0074. We compare this with the values with $\alpha_q/\alpha_p = 1$, which were $P/Q = 0.075$ and 13.2. These give $\overline{P} = 0.93$ and 0.07 for the conducting and resting states, respectively.

In a channel passing $Na^+$ ions, for example, channels that are open in the resting state of the membrane result in expenditure of metabolic energy to maintain the ionic gradient across the membrane. Metabolic efficiency is thus increased by decreasing the ratio of the fraction of all channels open in the resting state to that in the conducting state. We see that by this measure, the efficiency is increased by a factor of 5.8 (0.07/0.93:0.0074/0.57) as a result of the slowing of the relaxation of the field in the $q$ (closed) state by a factor of 10, as compared with the relaxation in the $p$ (open) state. Thus for a membrane having the same conductance when excited, the resting metabolic energy expenditure due to $Na^+$ ion leakage is reduced by a factor of ~5.8; 63% more $Na^{++}$ channels are then required to obtain the same conductance. This should, however, not result in any appreciable penalty to the membrane.

Such experimental data as is available from asymmetry or gating currents indicate that the relaxtion time ratio may be of the order of 10, and this value will be used in all the calculations to be presented. Change of this ratio does not, however, significantly change the results of calculations, other than to somewhat reduce the maximum value of $\overline{P}$.

## Interrelation of Membrane Environment and Γ

The theory presented here shows that the sensitivity of a membrane to its environment can be highly dependent upon the electrochemical potential difference of a diffusable ionic species across the membrane. The environmental change, i.e., the stimulus may, and in fact in most cases will, itself affect the electrochemical potential.

This is most clearly the case for electrical stimulation. In the $Na^+$ channel, a depolarizing stimulus moves the membrane potential closer to the equilibrium (Nernst) potential for $Na^+$, and thus reduces the available electrochemical potential, and therefore Γ; this should reduce the membrane sensitivity as compared with a stimulus that does not affect the electrochemical potential. The opposite is true for the $K^+$ channel; here a depolarizing stimulus increases the electrochemical potential. Mechanoreceptors are an example of the case where the stimulus does not directly affect the available electrochemical potential.

In the simplified model of gating being considered, the direct action of a stimulus is to increase $k_q$ (for simplicity $k_p$ remains constant). Then referring back to Eq. 6 and the definition of $k_q$, we find

$$\delta \log k_q = ze \cdot \delta V_m/kT. \tag{25}$$

At the same time, the electrochemical potential difference across the membrane will be changed by the same amount; for the $Na^{++}$ channel, Γ is reduced when $V_m$ becomes less negative

$$\delta\Gamma = -ze \cdot \delta V_m/kT \tag{26}$$

so that, as a first approximation

$$\delta\Gamma = -\delta \log k_q. \tag{27}$$

By similar reasoning, for the $K^+$ channel

$$\delta\Gamma = \delta \log k_q. \tag{28}$$

## Sensitivity for Very Fast Gating

We will now consider in more detail a system where the gates cycle very quickly as compared with the field relaxation time, i.e., where $k_p$ and $k_q$ are very large compared with $f_p$ and $f_q$. This may approximate the effect of adsorbed ions such as $Ca^{++}$ without other types of gating being effective.

This case was already considered above, but with the randomness in the field neglected. This gave for first-order relaxation, Eqs. 18a and b. In this limiting case, we found that with first-order relaxation functions (or other functional forms resulting in three intersections of Eqs. 20 and 21), there will be an abrupt population shift between high and low $P/Q$ points of intersection, and thus an abrupt shift in membrane conductivity.

We will now include the effect of randomness in the field, that is, in γ. We return to Eqs. 11, adding Eq. 11a and b to obtain $J_s$, the net flow of members along the γ coordinate. The sum is written as

$$J_s = S\left(\frac{P}{S}f_p - \frac{Q}{S}f_q\right) - D_p\mathrm{d}\left(S\frac{P}{S}\right)/\mathrm{d}\gamma - D_q\mathrm{d}\left(S\frac{Q}{S}\right)/\mathrm{d}\gamma, \tag{29}$$

where $S$ is the probability density of a member being at coordinate γ

$$S = P + Q. \tag{30}$$

Eq. 19 may be written as

$$P/Q = k_e e^{\gamma}, \tag{31}$$

where $k_e = k_p/k_q$. Then Eqs. 30 and 31 give

$$P/(S - P) = k_e e^{\gamma} \tag{32}$$

or

$$P/S = k_e/(k_e + e^{-\gamma}) \equiv A \tag{33a}$$

$$Q/S = 1 - P/S \equiv 1 - A \tag{33b}$$

$$\frac{d\,P/S}{d\gamma} = k_e e^{-\gamma}/(k_e + e^{-\gamma})^2 \equiv A' \tag{34a}$$

$$\frac{d\,Q/S}{d\gamma} \equiv -A'. \tag{34b}$$

Thus

$$J_s = S[Af_p - (1 - A)f_q] - D_p(SA' + A\,dS/d\gamma) - D_q[S - SA' + (1 - A)\,dS/d\gamma]. \tag{35}$$

In the steady state, $J_s$ must be constant everywhere; since for very large or very small values of $\gamma$, $J_s$ must approach 0, it must everywhere be 0. Therefore, in the steady state Eq. 35 becomes

$$\frac{dS}{S\,d\gamma} = \frac{d\log S}{d\gamma} = \frac{Af_p - (1 - A)f_q + A'(D_p - D_q)}{AD_p + (1 - A)D_q}. \tag{36}$$

Eq. 36 is integrated over $S$ as a function of $\gamma$; the integration constant is determined by the normalization condition

$$\int_{-\infty}^{\infty} S\,d\gamma = 1. \tag{37}$$

In practice, the integration need be performed only over a range of $\gamma$ sufficiently beyond 0 and $\Gamma$ to give very small values of $S$ at the limits of integration. Having thus found $S(\gamma)$, $P(\gamma)$ is calculated using Eq. 33a. Then

$$\int_{-\infty}^{\infty} P\,d\gamma = \bar{P},$$

the fraction of all channels that are open. The fractional conductivity of the membrane may, however, be higher than $P$, if in nominally closed channels the ion flow is not completely blocked. This correction may be of importance in calculating the blocking effect of some adsorbed species (13).

The free energy required to remove an adsorbed ion from the channel will include several terms. The electrostatic energy term, $\Delta G_e$, is given by

$$\Delta G_e = -ze\Delta V, \tag{38}$$

where $\Delta V$ is the electrostatic potential difference binding an ion of valence $z$ to the channel. There will, in general, also be a specific or chemical binding energy, $\Delta G_s$, which is independent of $\Delta V$ (13). The sum of these two terms gives $\Delta G_{ad}$, the adsorption free energy. This, along with the concentration of the adsorbed ion species, will determine $k_e$ (Eq. 31).

The energy effective in blocking by an adsorbed ion species will, however, include a third, activation free energy, term, $\Delta G_a$. This energy, equally affecting both the rate of adsorption and desorption, will not affect the value of $k_e$. It will, however, add to the energy required to remove an adsorbed ion from the mouth of the channel, and thus increase its blocking effect.

The total activation free energy effective in blocking flow through a channel is then

$$\Delta G^*_{ad} = \Delta G_e + \Delta G_s + \Delta G_a. \tag{39}$$

An adsorbed ion thus reduces the ion flow through a channel by the factor $\exp(-\Delta G^*_{ad}/kT)$, so $Q'$, the effective fraction of channels closed at coordinate $\gamma$, is

$$Q' = Q[1 - \exp(-\Delta G^*_{ad}/kT)]. \tag{40}$$

Then $P'$, the corresponding effective fraction of open channels, is

$$P' = S - Q' \tag{41}$$

and the fractional conductivity of the membrane is

$$\bar{P}' = \int_{-\infty}^{\infty} P'\,d\gamma. \tag{42}$$

The difference between $\bar{P}'$ and $\bar{P}$ is only significant for adsorption of species where $\Delta G^*_{ad}$ is small; for ions such as $Ca^{++}$, the value of $\bar{P}$ and $\bar{P}'$ are substantially equal. Therefore, $\Delta G_a$ need not be considered in calculating the steady state conductance.

## Steady State Response with Rapid Gating

Fig. 6 shows the calculated steady state response when gating is very rapid as compared with the first-order

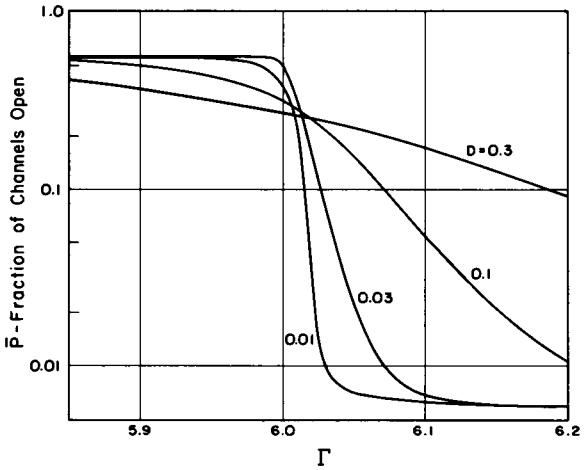


FIGURE 6 Steady state solution based on Eq. 36. Calculations assume $\Gamma$ is reduced equally with the energy holding the gate closed, so that $\delta\Gamma = \delta\log k_e$, as for the stimulation of the $Na^+$ process. The value of $D$ shown is $D/\alpha_p$, and varies with $\gamma$ as described in the text. First-order relaxation, with $\alpha_p/\alpha_q = 10$.

relaxation rate of the electric field (Eq. 9), as obtained by the use of Eq. 36. These curves are based on an $Na^+$ channel-like model, i.e., using Eq. 27, which here becomes

$$\delta\Gamma = -\delta \log k_e \quad (43)$$

since $k_p$ is assumed constant.

The randomness in the electric field is seen to have a very large effect on the sensitivity because this randomness is the only source of stochastic fluctuations in the model; as the randomness of the field approaches 0, the response approaches a step function. In this calculation $\alpha_p/\alpha_q = 10$. The labels of the curves are $D_p/\alpha_p$; as discussed in Appendix A, $D_q = D_p\alpha_q/\alpha_p$, and $D_p/\alpha_p = 0.03$, may be the order of magnitude for these parameters for the $Na^{++}$ channel in squid axon.

## Time-dependent Response with Very Fast Gates

The time course of the conductance change is obtained by the use of Eq. 35, not assuming $J_s = 0$. The continuity equation, $\partial S/\partial t = -\partial J_s/\partial\gamma$, becomes in finite form should be:

$$\Delta S(\gamma)/\Delta t = (J_s(\gamma + \delta\gamma/2) - J_s(\gamma - \delta\gamma/2]/\delta\gamma. \quad (44)$$

$\bar{P}$ is then calculated as described above for the steady state solution. The result of such a calculation for the same model as used in Fig. 6 is given in Fig. 7; the initial condition was $\Gamma = 6.1$, stepped to $\Gamma = 5.5$. For a divalent cation seeing the total change in electrochemical potential, this represents a voltage step of 7.5 mV. Fig. 7 demonstrates the important effect of randomness on the speed of response of a system having a bimodal distribution of $\gamma$, and thus the possibility of the appearance of two steady states.

As already mentioned, randomness results from both the finite time of the gating process and from the inherent fluctuations in the electric field. In the calculations of Fig. 7, the first source is eliminated by the assumption of infinitely fast gates. This then would result in channels remaining trapped in their initial distribution if there were no fluctuations in the electric field. Although this extreme case is purely theoretical, the calculations, Figs. 6 and 7 demonstrate a possible trade-off between membrane sensitivity and speed of response. For a system having relaxation functions that result in only a single point of intersection in Fig. 5, the results would be quite different.

## Sensitivity with Finite Gating Time

The steady state response of a system in which gating time is comparable with, or slower than the relaxation time of the electric field, is obtained by numerical integration of Eqs. 15; the numerical methods employed are given in Appendix B.

The effect of gating time in reducing the membrane sensitivity is shown by the results of such calculations as illustrated in Fig. 8. The labels on the curves are $k_p/\alpha_p$, i.e., the ratio of the relaxation time of the field when the gate is open $(1/\alpha_p)$ to the mean open time of the gate $(1/k_p)$. The dashed curve shows the sensitivity predicted by classical theory for a model in which the stimulus affects only $k_q$. The calculations are based on $\alpha_p/\alpha_q = 10$, and $\Gamma = 6$. Randomness of the electric field is assumed negligibly small ($D_p = D_q = 0$). It is seen that sensitivity is somewhat increased even with the gate closing at half the rate of open-gate relaxation, and increases rapidly for more rapid gating.

Fig. 9 illustrates similar calculations, but for an $Na^+$-like channel, in which $\Gamma$ decreases with membrane stimulation (Eq. 24). This considerably reduces the sensitivity, so that here the classical sensitivity is approached when $k_p = \alpha_p$.

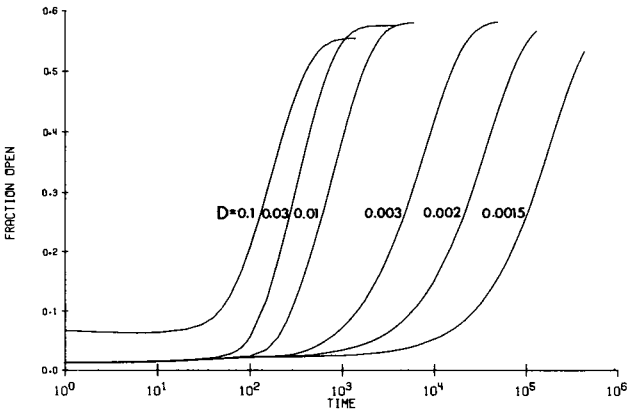


FIGURE 7 Time-dependent response of the system of Fig. 6, for a step from $\Gamma = 6.1$ to $\Gamma = 5.5$. Note that as $D$ becomes very small, the response time rapidly increases.

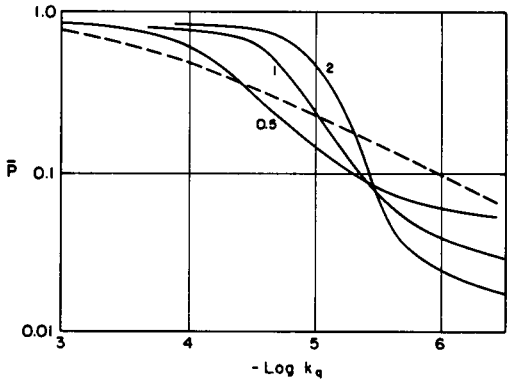


FIGURE 8 Effect of the ratio of mean open time of gate to relaxation time of the field when the gate is open. Dashed line (---) shows the change in permeability that would occur if the stimulus energy change acted directly on the gate (classical theory). Relaxation of electric field when the gate is closed is assumed to be ten times slower than when open.

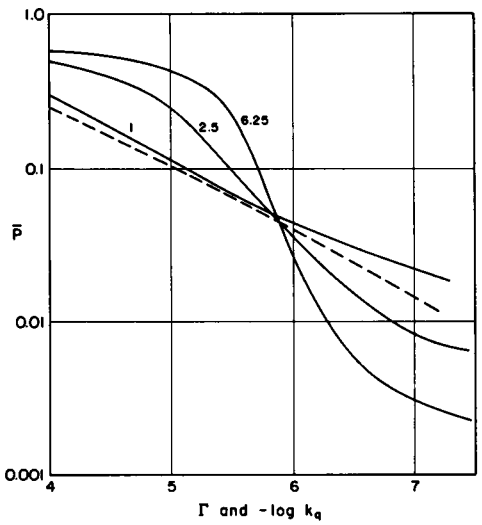


FIGURE 9 Same conditions as Fig. 8, except that Γ is assumed to be reduced equally with log $k_q$, as may approximately represent the effect of electrical stimulation of the $Na^+$ channel.

## THE DUAL EFFECT OF $CA^{++}$ WITH CONFORMATIONAL GATING

A variety of membranes that have now been studied by the patch-clamp technique (16–19) show evidence of conformational gating, with gate cycle times in the millisecond range. The macroscopic currents in these membranes are, in general, about an order of magnitude slower than in axon; but even if the gating process in axon is proportionately more rapid, the results given in Fig. 9 indicate that such conformational gating would not, of itself, result in the high sensitivity of excitable membranes.

Most, if not all, excitable membranes require the presence of divalent cations, usually $Ca^{++}$ in the external medium. It was shown above that the adsorption of an ion such as $Ca^{++}$ at the mouth of the channel under suitable conditions can be very sensitive to the membrane voltage. Accordingly, we will now consider a system in which both conformational gating, which may be rather slow, and rapid-cycling adsorption gating as by $Ca^{++}$ are operative. Anticipating the results, we will find that $Ca^{++}$ in effect can catalyze the conformational gates; the voltage (or other environmental) sensitivity of the $Ca^{++}$ adsorption is reflected in increased sensitivity of the conformational gates.

## ANALYSIS OF $CA^{++}$ EFFECT ON CONFORMATIONAL GATING

In the analysis, we start with Eqs. 11, with Eq. 11a applicable when the channel is completely unobstructed and Eq. 11b when it is fully closed. Thus Eq. 11b applies when the conformational gate is closed; this is, independent of the fractional time in which the channel may also be blocked by an adsorbed ion, because the conformational gate alone is assumed to result in complete blockage when closed.

We now focus our attention on the subset of only those channels having their conformational gates open. For this subset, which has the probability distribution $P(\gamma)$, we write a flow equation to give the net flow of members along the $\gamma$ coordinate due to $Ca^{++}$ adsorption. This equation, which now replaces Eq. 11a, will be the same as Eq. 21 except that the probability density of members in the subset is given by $P$, rather than $S$; and $P$ and $Q$, as used in Eq. 21, now refer to the probability densities of channels in this subset of conductive and of effectively blocked channels. Therefore, in Eq. 21 we replace $S$ by $P$, and we denote the probability densities of channels in the subset that are not blocked by adsorbed $Ca^{++}$ by $P_a$; and those that are blocked by $Q_a$. We then have

$$J = P\left(\frac{P_a}{P} f_p - \frac{Q_a}{P} f_q - D_p \mathrm{d}\frac{P_a}{P}/\mathrm{d}\gamma - D_q \mathrm{d}\frac{Q_a}{P}/\mathrm{d}\gamma\right) \tag{45}$$

with

$$P_a + Q_a = P. \tag{46}$$

$P_a/P$, $Q_a/P$, $\mathrm{d}(P_a/P)\mathrm{d}\gamma$, and $\mathrm{d}(Q_a/P)\mathrm{d}\gamma$ are all the same as their corresponding terms in $S$, Eqs. 33 and 34, so that Eq. 45 may be written as

$$J_p = P[Af_p - (1 - A)f_q] - D_p(PA' + A\mathrm{d}P/\mathrm{d}\gamma) - D_q[-PA' + (1 - A)\mathrm{d}P/\mathrm{d}\gamma] \tag{47}$$

and $J_q = -Qf_q - D_q \mathrm{d}Q/\mathrm{d}\gamma$ (already given as Eq. 11b).

We assume first-order kinetics, Eqs. 9; apply the continuity equation as in Eqs. 12 to obtain the rate of change of local probability densities due to flow; and add to this the rate of change due to the $p$-$q$ reaction, Eqs. 13. This then gives

$$\begin{aligned}\partial P/\partial t = &-[A\alpha_p(\Gamma - \gamma) - (1 - A)\alpha_q\gamma]\partial P/\partial\gamma \\ &-[A'\alpha_p(\Gamma - \gamma) - A\alpha_p + A'\alpha_q\gamma \\ &- (1 - A)\alpha_q]P + D_p(2A'\partial P/\partial\gamma + A''P \\ &+ A\partial^2 P/\partial\gamma^2) - D_q[2A'\partial P/\partial\gamma + A''P \\ &- (1 - A)\partial^2 P/\partial\gamma^2] - k_p P + k_q e^{\gamma} Q.\end{aligned} \tag{48}$$

$A'' = \mathrm{d}A'/\mathrm{d}\gamma$. The $Q$ equation (Eq. 16b) remains unchanged. The steady state solution is obtained as before by setting the time derivatives in Eqs. 16b and 48 equal to 0; the equations are solved by the numerical methods given in Appendix B. In this way, we obtain the probability density of channels having open gates. $\bar{P}$, the fraction of all the channels having open gates, may then be obtained as before by integrating $P$ over all values of $\gamma$.

This, however, does not give the relative conductivity of the membrane channels, since the value of $P$ does not include the blocking effect of the adsorbed ions. What is required is $\bar{P}_a$, which is the integral of $P_a$ over all values of

$\gamma$. This is obtained by multiplying the values of $P$ as obtained above as a function of $\gamma$, by $P_a/P$, as given by Eq. 33a

$$\bar{P}_a = \int_{-\infty}^{\infty} P \cdot \frac{P_a}{P} d\gamma. \quad (49)$$

## RESPONSE WITH BOTH $CA^{++}$ AND CONFORMATIONAL GATING

Fig. 10 gives the results of calculations for $Na^{++}$ channels (i.e., $\delta\Gamma$ related to $\Delta V_m$ by Eq. 26) with gating by a univalent conformational gate, as well as by $Ca^{++}$. The same voltage change is assumed to be effective on the conformational gate as on the adsorption of $Ca^{++}$. In these calculations, $\alpha_p$, the relaxation rate of the field with open gates, is 2.5 times $k_p$, the rate constant of gate closing. With these parameters and in the absence of $Ca^{++}$, the voltage sensitivity of the conformational gates (dotted curve) is no greater than would be predicted by classical theory. In the presence of external $Ca^{++}$, however, the voltage sensitivity of the gates is much higher (solid line curves). The voltage sensitivity of the conductivity is further increased at higher values of polarization by the additional gating effect of the $Ca^{++}$ itself; this is shown by the dashed curve, which shows the effective conductance resulting from the combined effect of the two gating processes. This curve does not depart appreciably from the conformational gate curve (solid line) except for lower values of $P$, indicating that the conductivity of most channel openings should be unaffected by $Ca^{++}$ concentration; its effect is rather on the number of channel openings.

$V_{dl}$, as shown in the figure, is the double-layer potential, i.e., the electrostatic potential binding the $Ca^{++}$, when $\gamma = 0$. The change in $V_{dl}$ is equal to the change in the voltage across the membrane. The binding energy for $Ca^{++}$ is assumed to also include a specific binding energy $\Delta G_s = 4$ kT (2.5 kcal/mol); the same results would be obtained with $\Delta G_s = 0$, but adding $-50$ mV to $V_{dl}$. Other parameters for the calculation are given in the legend to Fig. 10.

## DISCUSSION

The coupling of $Ca^{++}$ adsorption to conformational gating may be explained in nonmathematical terms. Consider a relatively slow conformational gating process, that is, $k_p$ smaller than $\alpha_p$. Then, in the absence of $Ca^{++}$, the gating sensitivity will approach that predicted by classical theory.

When the conformational gate is open, the effect of $Ca^{++}$ will be essentially as illustrated in Fig. 6. Consider a channel in which the (slow) conformational gate has been closed for a sufficient amount of time so that the electric field across the gate approaches its maximum value; i.e., $\gamma \rightarrow 0$. We now consider what happens when the gate

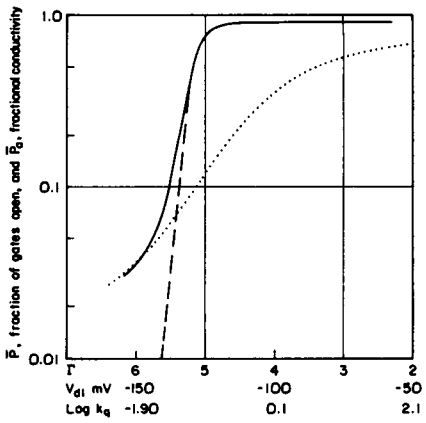


FIGURE 10 Steady state response of $Na^+$-like channels ($k_q$ increases with decrease in $\Gamma$), including $Ca^{++}$ effect on conformational gates carrying two charges. Electric field relaxes more rapidly than gates: $\alpha_p = 5$, $\alpha_q = 1$; $k_p = 2$, $k_q = 0.1$. $\Gamma = 6$ at resting potential (75 mV available electrochemical potential was available to both conformational gate and $Ca^{++}$). $V_{dl} = 125$ mV at resting potential. Upper solid curve (——): $\bar{P}$, the fraction of conformational gates open. Dashed line (---): effect of membrane potential on conformational gates with the same rate constants (except $k_p = 10$), but in absence of $Ca^{++}$. Note increase in voltage sensitivity in presence of $Ca^{++}$. Lower solid-line curve (——): overall relative conductivity of membrane, $\bar{P}_a$. Randomness constant $D = 0.03$ in this and following figures.

opens under conditions (membrane potential and $Ca^{++}$ concentration) such that the $\bar{P}_a$ (the fractional time the channel is not blocked by $Ca^{++}$) is low; i.e., the membrane potential is greater than its critical value. Under these conditions, even though the conformational gate is open, the channel remains effectively closed by $Ca^{++}$; the field across the gate will then not appreciably relax towards a lower value, and $\gamma$ will remain low. Thus when the gate again closes, it tends to remain closed ($\bar{P} \rightarrow 0$).

The opposite may occur if the membrane potential is reduced below its critical value, so that $\bar{P}_a \rightarrow 1$. In this case, when the gate opens the field relaxes, so that $\alpha \rightarrow 1$ at the rate given by $\alpha_p$. Thus when the conformational gate closes, it will remain closed a shorter time, and $\bar{P}$ will progressively increase — a more physical explanation of the catalytic effect of $Ca^{++}$ predicted by the analytic analysis.

The theory permits predictions concerning the effect of $Ca^{++}$ on the single channel conductance of $Na^+$ channels, as measured by patch-clamp experiments. As seen in Fig. 10 for the conditions of that calculation, for a depolarization of more than 25 mV from resting potential, the fractional conductance is substantially equal to the fraction of the conformational gates that are open. This implies that almost every channel opening will exhibit full conductivity, and the channel conductivity will be almost independent of $Ca^{++}$ concentration, although the membrane conductance will be strongly affected due to the effect of $Ca^{++}$ on the number of conducting channels.

## $Na^+$ and $K^+$ Channels

Fig. 11 gives the calculated conductivity as a function of membrane voltage, for one model of the $Na^+$ channel ($\Gamma$ decreasing with depolarization), with no inactivation. Results are given for three values of $Ca^{++}$ concentration. The Hodgkin-Huxley $m^3$ parameter is also plotted for comparison. Fig. 12 shows similar calculations for the $K^+$ channel ($\Gamma$ increasing with depolarization); here the $n^4$ parameter is also plotted. Parameters used for these calculations are given in the legends to the figures.

## What is the Nature of the $Na^+$ Channel?

Although the theory presented here appears to be theoretically sound from the standpoint of thermodynamics and statistical mechanics, is it of significance as applied to the $Na^+$ channel? The central question to be answered in this regard is that of the relaxation time of the electric field within the channel as compared with the mean dwell time of the gates, both conformational and ionic (i.e., $Ca^{++}$). If the relaxation time is very short compared with the dwell times, the the calculations presented, although correct, could have little importance for the $Na^+$ channel.

The charge motion associated with gating currents is equivalent to approximately three electron charges moving across the membrane per $Na^+$ channel. From single-channel experiments, as well as from estimates of channel density in membranes as made by various methods, the peak $Na^+$ current through a single channel is found to be of the order of $10^{-12}$A, or $6 \times 10^6$ ions/s. If gating current experiments are interpreted as showing that there are only

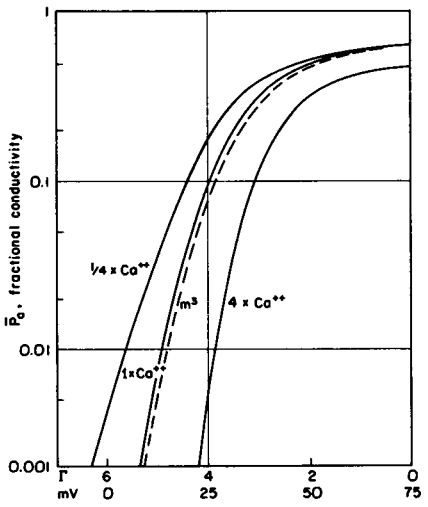


FIGURE 11 Steady state response of $Na^+$ channels showing effect of change of $Ca^{++}$ concentration. Parameters selected to approximate $m^3$ of the Hodgkin-Huxley axon. $\alpha_p = 10$, $\alpha_q = 1$; $k_p = 2$, $k_q = 0.01$. Other parameters same as Fig. 10. Three solid line curves are for normal $Ca^{++}$ concentration, 4 x, and 1/4 x $Ca^{++}$ concentrations. Dashed line (---) is 0.65 $m^3$.

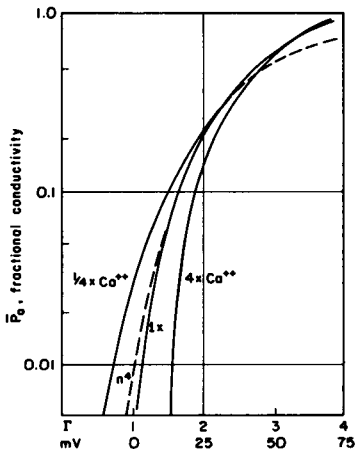


FIGURE 12 $K^{++}$ channel with $Ca^{++}$ effect. $\alpha_p = 50$, $\alpha_q = 5$; $k_p = 0.5$, $k_q = 0.05$. Singly charged gate. $\Gamma = 1$ at resting potential (25 mV lower than equilibrium potential). Other parameters same as Fig. 11. Dashed curve (--) is Hodgkin-Huxley $n^4$ parameter.

three mobile $Na^+$ ions present in a channel, the mean transit time of an ion in a channel would be $5 \times 10^{-7}$ s.

Such a short transit time would be difficult to reconcile with a field relaxation time of tens to hundreds of microseconds, as appears to be required by my theory; it is equally difficult to reconcile with the observed times of gating currents, if these are interpreted as due to the motion of ions. There is, however, no reason to believe that in fact the individual charges move completely across the membrane in gating experiments; rather, there may be many more charges involved, each moving but a small fraction of the distance across the membrane.

Present knowledge of the nature of the $Na^+$ channel is in fact more consistent with the latter view. It is now believed that the $Na^+$ channel exists in a protein of ~250,000 daltons in molecular weight (26). This implies the presence of ~2000 amino acid residues. Those derived from aspartic or glutamic acid will have carboxyl side chains; the *pK* of these amino acid residues is 4.4 in protein (27), so that at normal values of pH these groups will be ~99.8% ionized. Electrostatic forces will then require almost all these to have cations associated with them; in the $Na^+$ channel, most of these will be $Na^+$ ions.

As far as I am aware the proteins forming the voltage-sensitive $Na^+$ channel have not been sequenced. However, those for the acetylcholine-sensitive channel have been (28–30). In this channel the total of glutamic and aspartic residues in the $\alpha$, $\beta$, and $\gamma$ subunits is ~140, and it is reasonable to suppose that the two types of channels should not be vastly different.

Although all the carboxyl groups will not necessarily be available to the channel itself, it appears likely that the number of cations held in the channel by the ionized carboxyl groups will exceed a hundred. Thus an ion entering the channel at one interface could emerge from

the other only some tens of microseconds later; the relaxation time of the electric field with the opening or closing of the gate could be expected to be of the same order of magnitude.

## CONCLUSIONS

The main thrust of this work is that the energy available to control ionic flow through a membrane is not limited to that furnished directly by the applied stimulus, but by the total electrochemical potential difference existing across the membrane. The fraction of this electrochemical energy that can be mobilized by a stimulus depends upon a number of parameters, including the relaxation rates of the channel gates, the relaxation rates of the electric field within the channels, and the conductivity of an open channel, since this will affect the randomness of the electric field and thereby the sensitivity. Of particular interest to many workers in membrane physiology is the suggested influence of $Ca^{++}$ (or other divalent cations) on membrane sensitivity through its cooperative action on the channel gates.

Sodium inactivation has not been explicitly considered in the theory as here presented. Aldrich and Stevens (31) have recently shown that the $Na^+$ channel, in some cases at least, has three states: resting (closed), open, and inactivated; and have, by analysis of the single-channel statistics, been able to determine the rate constants of the transitions between states. This information now makes it possible to incorporate $Na^+$ inactivation into the present theory. Calculations based on such a model will be presented in a subsequent publication.

## APPENDIX A

### Estimate of the Stochastic Constant $D$

An estimate has been made of the value of $D$, based only on the thermal agitation component of the fluctuation voltage across the channel gates. The Einstein equation for charge fluctuation $\delta q$ in a conductor of conductance $G$ gives the mean square expected value in a time $t$ (24)

$$\langle \delta q^2 \rangle = 2GkTt. \tag{A1}$$

We take $R = 1/G$ to be the resistance of an open channel. Assume then that the voltage $V$ across the gate results from the charging of a capacitance $C$ through the channel resistance. Assuming first-order relaxation, $\alpha$, the relaxation rate constant of $V$ is then $\alpha = 1/RC$. We use here $\alpha = \alpha_q$, the rate constant for a closed channel, but use the open channel resistance under the assumption that most of the open channel resistance resides in the channel outside the gating region.

We may then rewrite Eq. A1 as

$$\frac{\langle \delta q^2 \rangle}{C^2} = \frac{2R}{R^2C^2} kTt$$

or

$$\langle \delta V^2 \rangle = 2R\alpha_q^2 kTt. \tag{A2}$$

Since we are interested in fluctuations in $\gamma$, i.e., the energy fluctuations of the gating process in units of kT, we multiply Eq. A2 by the square of the charge $q$ carried by the gate, and divide by $kT^2$

$$\langle \delta\gamma^2 \rangle = 2\frac{R\alpha_q^2 q_G^2}{kT} t \tag{A3}$$

Then the Einstein coefficient $D_q$ for the closed ($q$) state is given by

$$D_q = \frac{R\alpha_q^2 q_G^2}{kT} \tag{A4}$$

We assume that the rate constant in the $p$ state is higher because the capacitance is charged through a lower resistance, due to access from both sides of the channel. Then $R$ is lowered by the ratio $\alpha_q/\alpha_p$. This then gives

$$D_p = D_q \cdot \alpha_p/\alpha_q. \tag{A5}$$

Then the effective value of $D$ at any value of $\gamma$ is

$$D = \frac{P}{S} D_p + \frac{Q}{S} D_q \tag{A6}$$

with $P/S$ and $Q/S$ given by Eqs. 33a and 33b.

The conductance of a single $Na^+$ channel has been estimated by fluctuation noise analysis to be ~9 pS in frog node (32), and ~4 pS in squid axon (33). It has also been directly measured in single voltage-sensitive $Na^+$ channels, although not in axonal membranes. The values thus obtained were ~10 pS (18, 19). Assuming a conductivity of 10 pS, and $\alpha_q = 10^4\ s^{-1}$, Eq. A4 then gives $D_q$ ~250, and $D_p$ ~2,500, and $D_p/\alpha_p$ ~0.05.

## APPENDIX B

### Numerical Methods

Eqs. 15 or 16 or Eqs. 48 and 16b for the dual gate model have been integrated by the difference equation method. The integration has been performed both for the time-dependent solution and for the steady state by setting the time derivative equal to 0. Because of the randomness of $\gamma$, implied by a nonzero value of $D$, integration must be performed over an interval extending from $\gamma_{min}<0$, to $\gamma_{max}>\Gamma$; the amount by which the limits must be extended depends upon the values of the $D$s. The total interval from $\gamma_{min}$ to $\gamma_{max}$ is then divided into $N-1$ equal increments in $\gamma$, each of a width $h = (\gamma_{max} - \gamma_{min})/(N-1)$. A matrix of coefficients is then constructed, having $2N$ rows and columns. The rows contain alternately the numerical value of the coefficients for the equation giving the derivatives of $P$(Eqs. 14a, 15a, 16a, or 48), and those of $Q$ (Eqs. 14b, 15b, or 16b); the columns contain alternately the coefficients of $P$ and $Q$.

In evaluating the derivatives in $\gamma$ numerically, the three-point formulas are used for both first and second derivatives at all stations except the first and last

$$(dP/d\gamma)_i = (P_{i+1} - P_{i-1})/2h$$

$$(d^2P/d\gamma^2)_i = (P_{i+1} - 2P_i + P_{i-1})/h^2$$

and similarly for $Q$. At the first and last stations, only two points are available. At these stations, the two-point first derivative formula is used; e.g.,

$$(dP/d\gamma)_1 = (P_2 - P_1)/h,$$

$$(dP/d\gamma)_N = (P_N - P_{N-1})/h.$$

For the second derivative, I have found the most satisfactory approximation at the end stations is to employ the three-point formulas, but to assume $P_0 = Q_0 = P_{N+1} = Q_{N+1} = 0$. The error is minimized by the fact that the limits of integration are extended to the point that the values of $P$ and $Q$ are very small.

We then have a band matrix of coefficients consisting of the principal

diagonal with two upper and two lower diagonals. The elements of rows $2i$ and $2i + 1$ are then

$$X(P_{i-1}) \quad X(P_i)\ X(Q_i)\ X(P_{i+1})$$
$$Y(Q_{i-1})\ Y(P_i)\ Y(Q_i) \quad Y(Q_{i+1}).$$

The $X$ and $Y$ elements are, respectively, the coefficients in Eqs. 15a and b or their analogs; the $i - 1$ elements are of course omitted for $i = 1$, and the $i + 1$ element for $i = N$.

For the steady state solution, there will be no known terms in the equations. The vector of knowns thus becomes all 0, and the solution becomes indeterminate. It is therefore necessary to tie down the solution at some point; I have found it convenient to do so by setting the derivative $dP/d\gamma$ equal to a constant at one station; I have used the station at $\gamma = 0$, since $dP/d\gamma$ will be large in this region. The value of the constant is arbitrary, the final results being normalized so that

$$\int_{-\infty}^{\infty} (P + Q)d\gamma\ 1.$$

The band matrix is resolved by using IMSL subroutine LEQT1B.

The time-dependent equations are solved by setting up the matrix of coefficients in a similar manner, with the known values of $P$ and $Q$ calculated at the previous time step, as used in the vector of knowns; their initial values are obtained from the steady state solution. The parabolic partial differential solution is efficiently solved using the Crank-Nicholson method (34).

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