INFERENCES ABOUT MEMBRANE PROPERTIES FROM ELECTRICAL NOISE MEASUREMENTS

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ABSTRACT Four sources of electrical noise in biological membranes, each with a different physical basis, are discussed; the analysis of each type of noise potentially yields a different sort of information about membrane properties. (*a*) From the *thermal noise spectrum*, the passive membrane impedance may be obtained, so that thermal noise measurements are essentially equivalent to the type of sine wave analysis carried out by Cole and Curtis. (*b*) If adequately high frequency measurements could be made, the *shot noise spectrum* should give information about the average motion of a single ion within the membrane. (*c*) The number of charge carriers and single ion mobilities within the membrane can possibly be inferred from measurements of noise with a *1/f spectrum*. Available data indicate, for example, that increases in axon membrane conductance are not achieved by modulations in the mobility of ions within the membrane. (*d*) Fluctuations arising from the mechanisms normally responsible for membrane conductance changes can produce a type of electrical noise. Analysis of such *conductance fluctuations* provides a way to assess the validity of various microscopic models for the behavior of individual channels. Two different probabilistic interpretations of the Hodgkin-Huxley equations are investigated here and shown to yield different predictions about the spectrum of conductance fluctuations; thus, appropriate noise measurements may serve to eliminate certain classes of microscopic models for membrane conductance changes. Further, it is shown how the analysis of conductance fluctuations can, in some circumstances, provide an estimate of the conductance of a single channel.

INTRODUCTION

Measurements made on nerve and muscle membrane most usually yield average values of the variable under investigation. For example, in voltage clamp experiments on excitable membranes, the current-recording technique effectively averages individual currents flowing in a large population of channels. It is generally not possible, however, to infer the behavior of individual members of a population solely from such averages made over the entire population. Because of this limitation on making inferences from average behavior, a number of investigators have recently been studying electrical noise in nerve and muscle membranes in order to obtain additional information which may help reveal mechanisms operative at a more

microscopic level (Verveen and Derksen, 1965; Derksen, 1965; Derksen and Verveen, 1966; Verveen et al., 1967; Verveen and Derksen, 1968; Poussart, 1968; Verveen and Derksen, 1969; Poussart, 1971; Siebenga and Verveen, 1971; Verveen, 1971; Fishman, 1972; Katz and Miledi, 1970, 1971; Anderson and Stevens, 1972; see also Green and Yafuso, 1968, 1969; Yafuso and Green, 1971; Bean et al., 1969; Ehrenstein et al., 1970; Hladky and Haydon, 1970; DeFelice and Firth, 1971 *a*, *b*; DeFelice and Michalides, 1971). The purpose of the discussion here is to examine the sources for noise present in biological membranes and to indicate some of the inferences about membrane properties that can be made from measurements on electrical noise.

On the basis of physical theory, at least four sources for electrical noise should be present in nerve and muscle membrane:

(a) *Thermal (Johnson-Nyquist) noise.* Membrane potential and membrane current fluctuations arise from the thermal agitation of charge carriers, principally small ions.

(b) *Shot noise.* Because the passage of each ion through the membrane is analogous to the movement of electrons from the cathode to the anode in electron tubes, a type of electrical noise analogous to the shot effect in these devices should be present.

(c) $1/f$ *(Flicker) noise.* Although the precise physical basis is not at present clear, electrical noise generally is associated with the flow of current in systems with only a relatively small number of available charge carriers; thus, the presence of noise with a $1/f$ spectrum, familiar in other physical systems, might be expected in the nerve membrane. Such noise was discovered in the frog node (Verveen and Derksen, 1965) and has also been observed in lobster axon (Poussart, 1971) and squid axon (Fishman, 1972).

(*d*) *Conductance fluctuations.* To the extent that the mechanisms underlying membrane permeability changes are probabilistic, as they must be at some level, the membrane conductance must fluctuate. For example, if channels are envisioned as having only two states, open and closed, one could suppose that individual channels might be opening and closing at random, although the average number of channels open might remain constant. Alternatively, if an individual channel has a continually graded conductance from some minimum value to its maximum, that conductance presumably would fluctuate somewhat around its mean value so that, with all channels taken together, a net conductance fluctuation would occur. Electrical noise would result, then, from current flow through this fluctuating conductance; the existence of such conductance fluctuations has been proposed for end plate (Katz and Miledi, 1971) and axon (Siebenga and Verveen, 1971) membranes.

Each of the four noise sources indicated above should, in principle, contribute to the total electrical noise measured for a nerve membrane, and one might expect, in general, that the sources would be independent, although correlations might be

present in particular cases. For example, if the conductance fluctuations were very large, the other noise sources would also reflect the nonconstant conductance.

Because each of these noise sources has a different physical basis, the analysis of each gives, as will be indicated in greater detail below, a different sort of information about the nerve membrane. From the thermal noise spectrum, the passive membrane impedance may be inferred. If adequately high frequency measurements could be made, the shot noise spectrum should give information about the average motion of a single ion within the membrane. The number of charge carriers and single ion mobilities within the membrane can possibly be inferred from the amplitude of the $1/f$ noise. Finally, analysis of conductance fluctuations provides a way to assess the validity of various microscopic models for behavior of individual channels and may make it possible to estimate quantities such as the conductance of a single channel. In the succeeding discussion, each of the four noise sources will be examined, the first three sources briefly, and the conductance fluctuations in greater detail.

NOTATION

$S_j(f)$	Voltage spectral density for fluctuations arising from the jth source.
$W_j(f)$	Current spectral density for fluctuations arising from the jth source.
$M_j(f)$	Conductance spectral density for fluctuations arising from the jth source.
j	Source.
1	Thermal noise.
2	Shot noise.
3	Flicker noise.
4	Conductance fluctuation noise.

THERMAL NOISE

Thermal voltage noise is known to have a spectral density proportional to the real part of the membrane's complex passive impedance (see, for example, Bennett, 1960). Thus,

$$S_1(f) = 4kT \operatorname{Re} Z(f), \tag{1}$$

where $S_1(f)$ is the spectral density of voltage noise, $Z(f)$ is the complex impedance of the membrane, f is the frequency, and k and T are the Boltzmann constant and the absolute temperature. If voltage is held constant and the membrane current is measured, current noise with a spectral density $W_1(f)$ is given by

$$W_1(f) = 4kT \operatorname{Re}\left(\frac{1}{Z(f)}\right). \tag{2}$$

For a membrane represented by a simple parallel RC circuit, then, the noise voltage would have a spectral density

$$S_1(f) = \frac{4kTR}{1 + 4\pi^2(RCf)^2}, \tag{3}$$

and the current noise would have a spectral density

$$W_1(f) = \frac{4kT}{R}, \tag{4}$$

where R and C are the membrane resistance and capacitance respectively. Measurements of $S_1(f)$ and equation 3 would, for example, yield estimates of R and C, and further could be used to confirm that the membrane is represented by a parallel RC circuit.

Because the nerve membrane is not in fact accurately represented by an ideal parallel resistance and capacitance (see Cole, 1968), the voltage and current noise spectral densities given in equations 3 and 4 are only approximate representations of the actual spectral densities. From data for the membrane impedance of squid axon, equations 1 and 2 may be used to make a more accurate prediction of the thermal noise spectral densities (see Appendix A).

Although measurements of thermal noise spectral density yield, according to equations 1 and 2, only the real part of the complex impedance, the entire complex impedance can in principle be determined by noise measurements because it is possible to calculate the imaginary component of impedance from the real components: according to the Kramers-Kronig dispersion relations (see, for example, Mathews and Walker, 1965), the imaginary part of the complex impedance is, for a two-terminal passive network, simply the Hilbert transform of the real part. Thus, measurements of thermal noise spectra can, in principle, give the entire complex impedance of the nerve membrane and are thus equivalent to the sine wave analyses done by Cole and Curtis (see Cole, 1968).

SHOT NOISE

Let $F(t)$ denote the current which flows through the measuring circuit as the result of a single ion moving through the membrane. If it is assumed for simplicity that each ion has approximately the same motion within the membrane, that ions move independently, and that they enter the membrane according to a Poisson process with a rate r, then, according to shot noise theory (see Rice, 1954), the spectral density $W_2(f)$ for the noise current from this source, under voltage clamp conditions, should be given by

$$W_2(f) = r\,|\,\mathfrak{F}\{F(t)\}\,|^2. \tag{5}$$

The $\mathfrak{F}$ here indicates a Fourier transform. Under current clamp conditions, the voltage noise spectrum $S_2(f)$ is given by

$$S_2(f) = W_2(f)|\,Z(f)|^2, \tag{6}$$

since the membrane complex impedance $Z(f)$ filters the current noise; it should be

noted that equation 6 is a general relationship between the voltage and current noise spectra.

Because the shot noise current spectrum is related to $F(t)$, which in turn reflects the ion motion within the membrane, measurements of $W_2(f)$ can in principle yield a picture of the passage of ions through the membrane. In practice, however, $W_2(f)$ is probably unmeasurable because the spectral density would have to be determined accurately to extremely high frequencies. The requirement for high frequency measurements arises from the fact that a single ion transit through the membrane should cause only a brief current flow, which in turn implies that the Fourier transform of this function spreads over a very large frequency range.

As a specific simple example, if an ion moves with a constant velocity through a membrane with a uniform dielectric constant, $F(t)$ is given by

$$F(t) = \frac{q}{\bar{t}} (U(t) - U(t - \bar{t})),$$

where q is the ionic charge magnitude, $\bar{t}$ is the average transit time through the membrane, $U(t)$ is the unit step function, and the ion is assumed to have entered the membrane at $t = 0$. For this example, $W_2(f)$ is, according to equation 5, given by

$$W_2(f) = r \mid \mathfrak{F}\{F(t)\} \mid^2 = 2r \left(\frac{q}{2\pi f \bar{t}}\right)^2 (1 - \cos 2\pi f \bar{t}).$$

When f is small compared with $1/\bar{t}$, the spectral density is approximately constant and is given by

$$W_2(f) = r \left(\frac{q}{2\pi \bar{t}}\right)^2, \qquad f \ll \frac{1}{\bar{t}}.$$

If, as an example, the mobility of sodium ions within the membrane is assumed to be the same as that of sodium ions in water, the transit time of a sodium ion through a 100-A-thick membrane with a driving voltage of 10 mv would be about 0.2 μsec. Thus, the spectral density would be approximately constant to frequencies greater than about 0.5 MHz for a 10 mv driving voltage; in general, the cutoff frequency depends on membrane potential through $\bar{t}$. Even if the mobility of ions within the membrane is much less than that in water, it is clear that very high frequency measurements would be required to fully characterize $W_2(f)$.

Because $W_2(f)$ is probably unmeasurable, deficiencies in the shot noise model which led to the expression for this spectral density are not especially alarming. If it happened that a shot noise-like spectrum were in fact observable in the nerve membrane, a more refined model which allowed for interactions between current-carrying ions might well be required.

FLICKER NOISE

Although the physical basis of $1/f$ noise is not yet understood, Hooge (1969) and Hooge and Gaal (1971) have proposed an empirical law which relates this noise to relevant physical factors. According to Hooge's law, the spectral density for current noise measured under voltage clamp conditions is proportional to the square of the mean current, and inversely proportional to the number of charge carriers as well as to the frequency; thus

$$W_3(f) = \frac{A}{N_T}\frac{I^2}{f}. \tag{7}$$

$W_3(f)$ is the spectral density for current noise, N_T is the total number of charge carriers within the membrane, I is the mean current, f is the frequency, and A is a constant equal to about 2×10^{-3} for electrons in metals (Hooge, 1969) and about 1 for ions in 0.1 M salt solutions (Hooge and Gaal, 1971). As before, with current clamp, the voltage spectral density $S_3(f)$ is related to $W_3(f)$ by

$$S_3(f) = W_3(f)|\, Z(f)|^2.$$

According to Hooge's law, the $1/f$ noise spectrum behaves as if it were produced by conductance fluctuations given by

$$M_3(f) = \frac{A}{N_T}\frac{g^2}{f}, \tag{8}$$

where $M_3(f)$ is the spectral density of conductance fluctuations, and g is the average conductance of the mechanism in question. Conductance is related to the ionic mobility by

$$g = quN_T. \tag{9}$$

Here q is the single ionic charge magnitude and u is the average mobility per ion for the channel with a fixed length. Substituting this relationship into equation 8 gives

$$M_3(f) = \frac{Augq}{f}. \tag{10}$$

which may be solved for the single ion average mobility

$$u = \frac{M_3(f)f}{Agq}. \tag{11}$$

Assuming that Hooge's law may be applied to the nerve membrane, then, equation 11 yields the average single ion mobility as a function of conductance from measurements of $M_3(f)$. If, for example, membrane conductance is altered by some mecha-

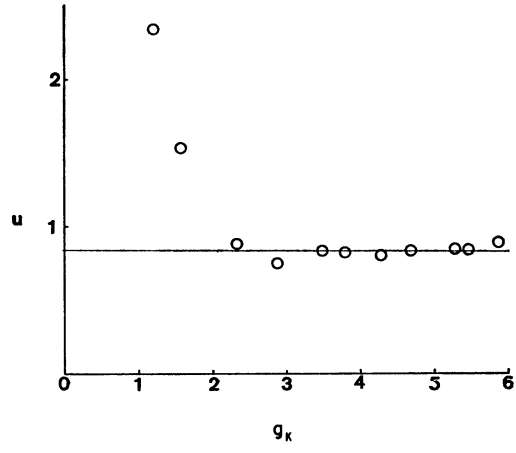

FIGURE 1 Single ion mobility u for a potassium channel as a function of potassium conductance g_K. Data points were calculated from Poussart's (1968) data for preparation D54.5 by means of equations 11. Mobility is obtained only up to a multiplicative constant because effective channel length is unknown; mobility units are thus arbitrary. Potassium conductance is given in reciprocal megohms.

nism which changes ionic mobilities within the membrane, this mechanism may be studied through the use of equation 11; alternatively, if conductance increases occur by opening larger numbers of channels with only two states, the single ion mobility estimated by equation 11 should be approximately constant.

Poussart (1968) has presented data (preparation D54.5) which permit average potassium ion mobility to be calculated from equation 11 for voltage-clamped lobster axon. Average mobility u (up to a multiplicative constant) as a function of the potassium conductance g_K is presented in Fig. 1; g_K in this figure varies over most of the range from 0 to $\bar{g}_K$. Because mobility is approximately constant except for the lowest potassium conductance values, the simplest interpretation of these data is that mobility is in fact constant for all values of potassium conductance, and that the two spuriously high mobility values resulted from contamination of the small potassium noise currents with, for example, noise currents through leakage channels. In any event, the data presented in Fig. 1 are not consistent with a model in which potassium conductance increases by a voltage-sensitive increase of potassium ion mobility within the membrane.

If A in equation 7 were known, it would be possible to calculate the number of ions that carry current; further, using the Hodgkin and Keynes (1955) estimate of two to three potassium ions per channel, an approximate value of the single channel conductance could be obtained. Unfortunately, it is especially difficult to extrapolate the value of A from the system studied by Hooge and Gaal (1971) because A is concentration dependent; the notion of channel ion concentration is, of course, ill-defined.

CONDUCTANCE FLUCTUATIONS

Conductance fluctuations in nerve and muscle membrane arise when the underlying mechanisms which determine membrane conductance are inherently probabilistic. For example, Katz and Miledi (1971) have proposed that, at the end plate membrane, acetylcholine molecules interact at random times with receptor molecules, the rate of interaction being dependent upon acetylcholine concentration, to induce a rapid rise and exponential decay of elementary end plate conductances. On this view, then, the end plate conductance mechanism is described by a random process formally like the shot noise type discussed earlier. Similarly, for electrically excitable membranes, one could view individual channels opening and closing randomly at rates which give a constant average conductance with superimposed small fluctuations.

Depending on which microscopic models of conductance mechanisms are selected, different types of noise spectra can arise from conductance fluctuations; indeed, one of the possible contributions of noise measurements is to aid in distinguishing between various underlying mechanisms. In the following discussion, two different models, both based on the Hodgkin-Huxley (1952) equations, will be considered, and these two different interpretations of the Hodgkin-Huxley equations will be seen to yield different noise spectra. After electrically excitable membranes have been discussed, there will be a brief consideration of models for end plate noise.

First Interpretation of Hodgkin-Huxley Equations

For clarity, the probabilistic version of the Hodgkin-Huxley equations will be presented here in terms of a specific physical model which follows the spirit of Hodgkin and Huxley's original picture and which seems adequate to account for at least one type of channel voltage sensitivity (Magleby and Stevens, 1972); other physical interpretations can, of course, be given to the equations presented here. Further, the Hodgkin-Huxley potassium channels will be treated, although a generalization to other channel types is obvious and immediate. Each channel is viewed as being guarded by four macromolecules that can change their conformation and block the channel. If any one of the molecules blocks a channel, then potassium ions cannot pass through; if all macromolecules are in their open conformation, then the channel is open and has a conductance γ (the closed conductance is assumed to be zero). The rates of conformational change are voltage dependent and are given by the Hodgkin-Huxley rate constants α_n and β_n. It is assumed that each of the gate molecules can have only two conformations (open and closed). The following master equation (van Hove, 1957; Zwanzig, 1964) describes the probability $p(k \mid t)$ that, given a gate molecule in state k at time 0, the molecule is in the open conformation at time t:

$$\tau \frac{dp(k \mid t)}{dt} + p(k \mid t) = n_\infty . \tag{12}$$

Here

$$\tau = \frac{1}{\alpha_n + \beta_n},$$

and α_n and β_n have the same significance as in the Hodgkin-Huxley equation, as does n_∞ ; k may denote the open (o) or closed (c) conformation of the gate molecule. The probability $n(t)$ that a molecule is in its open conformation at time t, irrespective of its initial conformation, is given by

$$n(t) = p(o)p(o \mid t) + p(c)p(c \mid t), \tag{13}$$

where $p(k)$ is the probability that the gate molecule is in its kth conformation (open o and closed c) initially.

The channel may be open only if all gate molecules are in their open conformation, so that the probability a channel is open is, assuming the gate molecules do not interact, equal to n^4. The average conductance $g_K(V, t)$ at time t is the average number of channels open Nn^4 (where N is the total number of channels) times the conductance γ of an open channel:

$$g_K(V, t) = N\gamma n^4. \tag{14}$$

Equations 12, 13, and 14 thus provide a probabilistic model which is equivalent to the ordinary Hodgkin-Huxley equations.

Having formulated a probabilistic version of the Hodgkin-Huxley equations, we may now calculate the characteristics of conductance fluctuations which, according to this model, produce electrical noise. This calculation is most conveniently carried out by first obtaining the covariance function

$$C(t) = E\{\tilde{g}(o)\tilde{g}(t)\} - E\{\tilde{g}(\infty)\}^2,$$

where E denotes the expectation, and $\tilde{g}(t)$ is the conductance at time t; the covariance function is then Fourier transformed to yield the spectral density.

Because the covariance function for N independent channels is N times the covariance function $C_1(t)$ for a single channel, we need only the quantity

$$C_1(t) = E\{\tilde{g}_1(o)\tilde{g}_1(t)\} - E\{\tilde{g}_1(\infty)\}^2,$$

where $\tilde{g}_1(t)$ denotes the conductance of a single channel at time t. By the definition of expectation $E\{\tilde{g}_1(o)\tilde{g}_1(t)\} = \sum_{j,k} \lambda(j)\lambda(k)P(j, k; t)$. $P(j, k; t)$ is the joint probability of finding the channel in state j initially and state k at time t, and $\lambda(j)$ is the

conductance of a channel in the j state; j (and k) may be either open (o) or closed (c). If the open conductance is γ and the closed conductance is 0, that is, if $\lambda(o) = \gamma$ and $\lambda(c) = 0$, all terms in the above summation vanish except $P(o, o; t)\gamma^2$. Since the channel may be open only if all four gating molecules in the open conformation,

$$P(o, o; t) = [p(o)p(o \mid t)]^4.$$

Thus, the expectation is given by

$$E\{\tilde{g}_1(o)\tilde{g}_1(t)\} = \gamma^2[p(o)p(o \mid t)]^4,$$

and the single channel covariance by

$$C_1(t) = \gamma^2 p^4(o)p^4(o \mid t) - \gamma^2 p^8(o \mid \infty), \qquad t \geq 0.$$

Equation 12 may be solved for the conditional probability $p(o \mid t)$ to give

$$p(o \mid t) = e^{-t/\tau}(1 - n_\infty) + n_\infty .$$

In the stationary state, then, $p(o) = p(o \mid \infty) = n_\infty$ so that

$$C_1(t) = \gamma^2 n_\infty^4 (e^{-t/\tau}(1 - n_\infty) + n_\infty)^4 - \gamma^2 n_\infty^8, \qquad t \geq 0,$$

which gives, after expanding the fourth-power term,

$$C_1(t) = \gamma^2 n_\infty^4 \sum_{j=1}^{4} \binom{4}{j} n_\infty^{4-j} e^{-jt/\tau}(1 - n_\infty)^j, \qquad t \geq 0.$$

The covariance function $C(t)$ for the entire membrane containing N channels is

$$C(t) = NC_1(t),$$

which gives

$$C(t) = N\gamma^2 n_\infty^4 \sum_{j=1}^{4} \binom{4}{j} n_\infty^{4-j} e^{-1^{jt/\tau_1}}(1 - n_\infty)^j. \tag{15}$$

To calculate the spectral density of conductance fluctuations, it is necessary only to take the Fourier transform of the covariance function $C(t)$. Thus, the spectral density $M_4(f)$ is given by

$$M_4(f) = N\gamma^2 n_\infty^4 \sum_{j=1}^{4} \binom{4}{j} n_\infty^{4-j}(1 - n_\infty)^j \frac{2\left(\frac{\tau}{j}\right)}{1 + \left(2\pi f \frac{\tau}{j}\right)^2}. \tag{16}$$

The spectrum of conductance fluctuations is constant in the low frequencies and decreases according to $1/f^2$ in the high frequency limit, that is, for frequencies well above $2/\pi\tau$. Hill and Chen (1972) have obtained this same relationship, equation 16, from another, but formally equivalent, probabilistic version of the Hodgkin-Huxley equations by FitzHugh (1965).

Second Interpretation of the Hodgkin-Huxley Equations

An alternative derivation of the conductance fluctuation spectrum is based on a literal interpretation of the Hodgkin-Huxley equations in which the accessory variable n is treated as being subject to random fluctuations from an unspecified source. This treatment then makes use of a theorem, the fluctuation-dissipation theorem, adapted from statistical mechanics.

For many physical systems, the return toward equilibrium from spontaneous fluctuations and the relaxation from external perturbations follow the same time-course; systems whose behavior exhibits this property are said to conform to Onsager's hypothesis, and Kubo (1957) has demonstrated in his fluctuation-dissipation theorem that such a property follows from the statistical mechanical description of the response of systems to small perturbations. Although the fluctuation-dissipation theorem need not be applicable to some arbitrarily chosen response of a complex system, it can be shown that it is applicable to a system of the type described by the Hodgkin-Huxley equations if the state of the conductance mechanism is completely specified by the appropriate Hodgkin-Huxley accessory variable (n for example) (see Appendix B). Specifically, the linearized Hodgkin-Huxley equations can be used to predict the spectral density of spontaneous conductance fluctuations in the nerve membrane; if the relaxation of, for example, the potassium conductance to its steady-state value is described (for responses in the linear region) by the function $R(t)$, then the spectral density of conductance fluctuations $M_4'(f)$ is given by

$$M_4'(f) = K \operatorname{Re} \mathfrak{F}\{R(t)\}, \tag{17}$$

where K is a constant.

Attention will again be restricted to Hodgkin-Huxley-type potassium channels. For concreteness, n may be considered now as specifying the fraction that a gating molecule has rotated (between minimum closed and maximum fully open), and it will be supposed that the channel conductance is approximated by γn^4, with n described by the usual Hodgkin-Huxley rate equation; γ is the maximum channel conductance. The following treatment does not require this physical interpretation, and in fact holds for any model that treats n as completely specifying the state of a channel.

Let ν be a small deviation of n from its steady-state value $n(V, \infty)$, and ψ be the corresponding deviation of voltage from its steady value $\bar{V}$, so that

$$n = n(V, \infty) + \nu, \qquad V = \bar{V} + \psi.$$

To first order in voltage ψ, then the linearized Hodgkin-Huxley equations are

$$g_K(V, t) = g_K n^4(V, \infty) + 4g_K n^3(V, \infty)\nu,$$

$$\tau \frac{d\nu}{dt} + \nu = a\psi,$$

where a is a constant. According to these equations, the potassium conductance (under voltage clamp conditions) relaxes to its final value according to

$$\nu(\psi, t) = \nu(o)e^{-t/\tau}, \qquad \psi = \text{constant},$$

provided that $g_K(V, 0)$ is not very different from $g_K(V, \infty)$. From the fluctuation-dissipation-like theorem, equation 17, the spectrum of conductance fluctuations $M_4'(f)$ is thus (for a constant voltage) given by

$$M_4'(f) = \frac{2\sigma^2\tau}{1 + (2\pi\tau f)^2}, \tag{18}$$

where the possibly voltage-dependent constant σ^2 is not provided by this theory. Up to an undetermined multiplicative constant, then, the spectral density of conductance fluctuations may be calculated from a voltage clamp analysis on the nerve membrane in question. The current spectrum $W_4'(f)$ under voltage clamp is given by

$$W_4'(f) = M_4'(f)\bar{V},$$

where $\bar{V}$ is the difference between the membrane potential and the potassium equilibrium potential; $S_4'(f)$ is obtained from

$$S_4'(f) = W_4'(f)|\, Z(f)|^2.$$

Both of the probabilistic interpretations of the Hodgkin-Huxley equations discussed here give rise to conductance fluctuations with a spectrum that is flat in the low frequency region and decreases as does $1/f^2$ in the high frequency limit. The "corner frequency," that is the frequency at which the extrapolated $1/f^2$ decline intersects the low frequency limit, is different in the two models, however, as is apparent from equations 16 and 18. For the first model (equation 16), the corner frequency is $2/\pi\tau$, whereas for the second model (equation 18), it is four times lower, $1/2\pi\tau$. Furthermore, the precise shape of the transition between the low and high frequency limits is different in the two cases, but this effect would be more difficult to detect in experiments.

One potential use for spectra of conductance fluctuations, then, is to distinguish between the two types of model described here. On the one hand are the models

which basically interpret n as a probability that one of four components is in a given state, and on the other hand are the models which view n as a state variable—that is, as a single variable, the value of which specifies the state of each (independent) potassium channel—subject to continuous random fluctuations. By comparing the cutoff frequency of the noise specrum to the value of τ at that voltage obtained in a voltage clamp experiment, it may be possible to decide between these two classes of models. Siebenga and Verveen (1971) have reported the presence of $1/f^2$ noise in frog node and have attributed this to conductance fluctuations, and Fishman (personal communication) has confirmed the presence of a $1/f^2$ noise component in squid axon. The data so far reported, however, are insufficient to indicate clearly if either of the models described here is adequate.

It is appropriate to emphasize an important difference in the two approaches used here to calculate the spectral densities of conductance fluctuations. The first probabilistic interpretation is specific, and the spectrum that results holds only for the particular model used or for a formally equivalent one (e.g., FitzHugh, 1965). The second probabilistic interpretation is general, however, and depends only on the fact that the Hodgkin-Huxley equations are an adequate description of excitable membrane behavior and the assumption that the state of the conductance mechanism is completely specified by the accessory variable n. Thus, any physical picture in which a single variable completely specifies the state of the conductance mechanism would give rise to the spectrum given in equation 18. The derivation for the fluctuation-dissipation-like theorem given in Appendix B will not, in general, hold for more than one variable so that any model in which n does not completely specify the state of the conductance mechanism, as in the first probabilistic interpretation, for example, will generally give rise to a different spectral density for conductance fluctuations. If the experimental $1/f^2$ spectra do not conform to equation 18, then, one may attribute this departure from predicted behavior to an inadequacy in the Hodgkin-Huxley description or conclude that multiple "hidden" variables (as in the first interpretation, for example) are required to specify the state of the conductance mechanisms.

Other properties of channels can also be determined, in principle, at least, through measurements of conductance fluctuation spectra. For example, it is not at present clear whether individual channels have two states (open and closed), several states (closed, half open, fully open), or perhaps a continuously graded conductance over some range, although the evidence in Fig. 1 argues against this last alternative. The various possibilities will not be systematically investigated here, but it is important to note that each model gives a different spectral density for conductance fluctuations, and probably more importantly, for the variance of conductance fluctuations as a function of voltage. For example, because the variance is the integral over all of the spectral density, and because n_∞ is a known function of voltage, the variance for the two-state channel model which led to equation 16 is a specified function of voltage; for very negative membrane potentials the variance is zero, it increases with

depolarization to the voltage at which $n_\infty = 0.5$, and then decreases toward zero with further depolarizations. In general, different voltage dependence of the variance would arise from other models.

End Plate Conductance Fluctuations

As noted earlier, it is probable that conductance fluctuations occur at the end plate membrane during the action of iontophoretically applied acetylcholine (Katz and Miledi, 1970, 1971). A modification of the preceding development provides an alternative to the model proposed by Katz and Miledi (1971) and yields a prediction for the spectrum of the observed conductance fluctuations. Instead of treating the elementary conductance change at the end plate as a rapid increase followed by an exponential decline, as Katz and Miledi have done, one may view an end plate channel as opening at random and then remaining open for a random length of time. According to this view, the opening and closing of a channel is described by the same equation 12 that was used previously to characterize the two conformational states of the hypothetical gate molecules; an end plate channel is considered to have, in effect, only a single-gate molecule. An analysis similar to that given earlier leads to a spectral density $M_4^e(f)$ for end plate conductance fluctuations given by

$$M_4^e(f) = \frac{N\gamma a}{1 + (2\pi\tau f)^2}, \qquad (19)$$

where N is the number of receptors, γ is the conductance of an open channel, and a is the (steady-state) probability that a channel is open. This spectrum has the same form as that obtained from the shot noise model used by Katz and Miledi, and seems to account adequately for the presently available data (Katz and Miledi, 1971; Anderson and Stevens, 1972); as Katz and Miledi (1971) have suggested, measurements of noise spectra cannot be used to decide between the model they have proposed and that presented here.

One use of noise measurements at the neuromuscular junction, then, is to test models of the microscopic conductance changes which underlie the end plate conductance change. Thus current data (Katz and Miledi, 1971) are consistent with at least the two different microscopic models noted above, but these data serve to eliminate other microscopic mechanisms that might be proposed. An additional use of noise measurements, first proposed by Katz and Miledi (1971), is to estimate the magnitude of the unitary conductance change. Since, according to the model indicated above, channels have only two states (open and closed) and change between these states in a random fashion, the probability of having some particular number of channels open at a given time is governed by the binomial distribution. This implies that the average conductance μ_g is

$$\mu_g = \gamma N a,$$

and the variance in the conductance is

$$\sigma_g^2 = \gamma^2 Na(1 - a).$$

For appropriately small acetylcholine concentrations, the probability of a channel being open is small compared with 1, so that the variance is approximately

$$\sigma_g^2 \approx \gamma^2 Na = \gamma \mu_g .$$

Thus, the variance of conductance fluctuations is proportional to the mean conductance level (in the limit of low acetylcholine concentrations), with the proportionality constant being the conductance of one open channel. Since both the mean and variance of end plate conductance are measureable quantities, the conductance of a single open channel may (for the model described here) be estimated by their ratio:

$$\gamma = \frac{\sigma_g^2}{\mu_g} . \tag{20}$$

Adequate experimental data are not yet available to permit calculation of this quantity, but doubtless they soon will be.

Difficulty of Separating Spectra from Various Sources

The main practical limitation on making inferences about membrane processes from noise measurements is the difficulty in separating spectra from different sources. Not only must the four main spectral contributions be recognized, but also must various components to each type of spectrum be identified. For example, when membrane current is being carried by more than one type of ion, it is reasonable to assume that different ionic species will move in different ways within the membrane; thus, each ionic species would give rise to a shot noise spectrum, and the total shot noise spectrum would be, on the assumption that the various currents are independent, the sum of the individual spectra for separate ionic currents. As another example, consider conductance fluctuation noise in a Hodgkin-Huxley axon. The m, n, and h parameters all give rise to fluctuations, so that the total conductance fluctuation spectrum is the sum of contributions from these three components. By appropriate physiological maneuvers, for instance, studying the membrane in situations where sodium channels are essentially totally inactivated, it may be possible to separate out the various sources of conductance fluctuation spectra. The main point is, however, that separating spectral components is inherently difficult and must be approached experimentally.

APPENDIX

A. Thermal Noise Spectrum for a Squid Membrane with Nonideal Passive Properties

The nerve membrane passive electrical properties are frequently represented by a parallel resistance-capacitance circuit. Although the capacitance may for many purposes be approximated by an ideal capacitor, a more accurate representation of the membrane impedance requires a modification of the types indicated in Fig. 2 where an ideal resistor R_m is in parallel with a nonideal series resistor $R(f)$ and capacitor $C(f)$. If the frequency-dependent resistance and capacitance are described by

$$R(f) = R_o(2\pi f\tau)^{\alpha-1} \sin \frac{\alpha\pi}{2},$$

$$C(f) = \frac{C_o}{(2\pi f\tau)^{\alpha} \cos \frac{\alpha\pi}{2}}; \qquad (A\,1)$$

the impedance of the membrane is well described, provided membrane potential variations are restricted to ranges which do not materially affect the Hodgkin-Huxley parameters. The parameters R_o, C_o, τ, α are constants and f denotes frequency. It will prove convenient to define

$$a = \frac{C_o}{\cos \frac{\alpha\pi}{2}}, \qquad b = R_o \sin \frac{\alpha\pi}{2},$$

so that

$$2\pi fC\tau = a(2\pi f\tau)^{1-\alpha},$$

$$RC = \frac{ab}{2\pi f\tau}. \qquad (A\,2)$$

By straightforward algebraic manipulations, the real part of the impedance for the squid

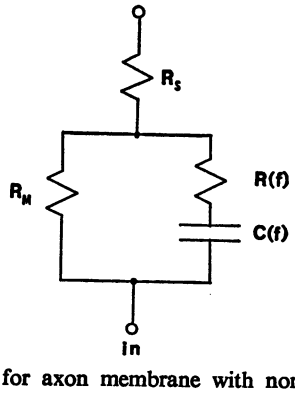

FIGURE 2 Equivalent circuit for axon membrane with nonideal capacitance. See Cole (1968) for a comprehensive review of passive membrane properties.

axon membrane, as represented by the circuit in Fig. 2, may be found. According to equation 1 of the text, then, the thermal noise voltage spectral density for a squid membrane under current clamp should be

$$S_1(f) = 4kTR_m \left(\frac{1 + \left(\frac{ab}{\tau}\right)^2 + \frac{a^2 b R_m}{\tau^2} (2\pi f\tau)^{1-\alpha}}{1 + \left(\frac{ab}{\tau} + \frac{aR_m}{\tau} (2\pi f\tau)^{1-\alpha}\right)^2} + \frac{R_s}{R_m} \right). \qquad (A\,3)$$

For the squid axon, $\alpha \approx 0.1$, so the spectral density should, in the high frequency limit, be approximately

$$S_1(f) \approx 4kT \left(\frac{b}{(2\pi f\tau)^{1-\alpha}} + R_s \right). \qquad (A\,4)$$

If R_s were sufficiently small, this mechanism would give noise with an approximately $1/f$ spectrum for high frequencies.

B. Fluctuation-Dissipation-like Theorem for Hodgkin-Huxley Model

If the random behavior of a probabilistic system is markovian, then it is true that the average future behavior of the system depends only on its present state (and not upon the path over which that state was approached), and a fluctuation-dissipation-like theorem is always valid. On the other hand, the fact that a system's *average* future behavior depends only on the present state, and not upon the past history, does not imply that the probability mechanism underlying the system's behavior is markovian. In this Appendix, it will be shown that a no-memory system, that is one whose *average* future evolution is determined only by its present state, need not be markovian, but that a fluctuation-dissipation theorem holds in any case. The Hodgkin-Huxley variables m, n, and h have their evolution determined completely by their initial values, so a fluctuation-dissipation theorem is valid for these subsidiary variables.

Let $P[\Phi(\tau)_{-\infty}^{0} \mid \xi, t]$ specify the probability density for finding a system in state ξ at time $t > 0$, conditional upon having followed a path $\Phi(\tau)$ from $-\infty$ up to time 0; $P[\cdot]$ is a function of ξ and a functional of Φ. It will be assumed that P is a continuous functional of Φ, and that the system is time homogeneous, so that a functional expansion may be employed:

$$P[\Phi(\tau)_{-\infty}^{0} \mid \xi, t] = P_0(\phi(0) \mid \xi, t) + \int_{-\infty}^{0} K(\xi, t - \tau)\Phi(\tau)\, d\tau + \cdots . \qquad (B\,1)$$

Although higher terms in the expansion have not been explicitly written, they have not been discarded. Let the functional $\mathbf{P}[\Phi(\tau)_{-\infty}^{0}]$ specify the probability density for following path $\Phi(\tau)$ and carry out the following functional integration over all paths which terminate at η at time 0:

$$\int_{\substack{\Phi: \\ \Phi(0)=\eta}} \mathbf{P}[\Phi] P[\Phi \mid \xi, t]\delta\Phi = P_o(\eta \mid \xi, t) \int_{\substack{\Phi: \\ \Phi(0)=\eta}} \mathbf{P}[\Phi]\delta\Phi + \int_{-\infty}^{0} K(\xi, t - \tau) \left(\int_{\substack{\Phi: \\ \Phi(0)=\eta}} \mathbf{P}[\Phi]\Phi\delta\Phi \right) dt + \cdots . \qquad (B\,2)$$

Because the probability density $P(\eta)$ for finding the system in state η at time 0 is given by

$$P(\eta) = \int_{\substack{\Phi: \\ \Phi(0)=\eta}} \mathbf{P}[\Phi]\delta\Phi ,$$

and the average path $f(\eta, \tau)$ which terminates at η is given by

$$f(\eta, \tau) = \int_{\substack{\Phi: \\ \Phi(0)=\eta}} \Phi(\tau)\mathbf{P}[\Phi]\delta\Phi ,$$

the functional integral above becomes

$$\int_{\substack{\Phi: \\ \Phi(0)=\eta}} \mathbf{P}[\Phi]P[\Phi \mid \xi, t]\delta\Phi = P(\eta)P_o(\eta \mid \xi, t) + \int_{-\infty}^{0} K(\xi, t-\tau)f(\eta, \tau)\, d\tau + \cdots . \quad (B\,3)$$

The effect of the above manipulations has been to characterize the probabilistic evolution of the system in terms of a markovian part given by $P(\eta)P_o(\eta \mid \xi, t)]$ and a non-markovian part characterized by the higher terms in the functional power series expansion.

The average relaxation of this system $\langle \xi(t) \rangle$ from its initial average position [characterized by $P(\eta)$] is by definition given by

$$\langle \xi(t) \rangle = \int_\eta \int_\xi \xi P(\eta)P_0(\eta \mid \xi, t)\, d\xi\, d\eta + \int_{-\infty}^{0} \bar{K}_1(t-\tau)\bar{f}(\tau)\, d\tau + \cdots \quad (B\,4)$$

where

$$\bar{f}(\tau) = \int_\eta f(\eta, \tau)\, d\eta \qquad \text{and } \bar{K}(t) = \int_\xi K(\xi, t)\xi\, d\xi .$$

For the average behavior for a system to exhibit no memory, it must be that the kernel $\bar{K}_1(t)$ vanishes, for $\langle \xi(t) \rangle$ must be independent of $\bar{f}(t)$ for every $\bar{f}(t)$; by a similar argument, the higher order kernels must also vanish. Thus, for a no-memory system,

$$\langle \xi(t) \rangle = \int_\xi \int_\eta \xi P(\eta)P_o(\eta \mid \xi, t)\, d\xi\, d\eta . \quad (B\,5)$$

The covariance of random fluctuations in $\xi(t)$ is by definition

$$\begin{aligned}\langle \xi(0)\xi(t) \rangle &= \int_\xi \int_\eta \xi\eta P_{eq}(\eta)P_o(\eta \mid \xi, t)\, d\xi\, d\eta \\ &\quad + \int_\xi \int_\eta \int_{-\infty}^{0} \xi\eta K(\xi, t-\tau)f(\eta, \tau)\, d\tau\, d\eta\, d\xi + \cdots \\ &= \int_\xi \int_\eta \xi\eta P_{eq}(\eta)P_o(\eta \mid \xi, t)\, d\xi\, d\eta \\ &\quad + \int_{-\infty}^{0} \int_\eta \eta\bar{K}(t-\tau)f(\eta, \tau)\, d\tau\, d\eta \cdots \quad (B\,6)\end{aligned}$$

where $P_{eq}(\eta)$ gives the equilibrium initial probability density; but for a process whose average behavior exhibits no memory, $\bar{K}_1(t) = 0$ (as do higher kernels), so that

$$\langle \xi(0)\xi(t) \rangle = \int_\xi \int_\eta \xi\eta P_{eq}(\eta) P_o(\eta \mid \xi, t) \, d\eta \, d\xi . \qquad (B\,7)$$

Because only the markovian part of the description survives in equations B 5 and B 7, it follows that, up to a multiplicative constant k,

$$\langle \xi(0)\xi(t) \rangle = k \langle \xi(t) \rangle .$$

This is a statement of the fluctuation-dissipation theorem.

Note that the random process need not be markovian; lack of memory macroscopically implies that $\bar{K}_1(t)$ vanishes (on the time scale of experimental analysis of the system), but the kernel $K(\xi, t)$ which describes departures from markovian behavior does not necessarily vanish. The fact that $\bar{K}(t) = \int_\xi \xi K(\xi, t) \, d\xi = 0$ does not necessarily mean that $K(\xi, t)$ is itself zero.

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