ON THE THEORY OF ION TRANSPORT ACROSS THE NERVE MEMBRANE

IV. NOISE FROM THE OPEN-CLOSE KINETICS OF K^+ CHANNELS

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ABSTRACT The theoretical power density spectrum $G(f)$ of fluctuations in the steady-state squid axon K^+ current in the 10^3 Hz region has been derived assuming that these are fluctuations in the number of open K^+ channels in the Hodgkin-Huxley (HH) model. Various modifications of the HH model were also studied. The results were negative in all cases when compared with experiment. This confirms the generally held view that the observed $G(f) \sim 1/f$ is associated primarily with K^+ current through open K^+ channels and not with the open-close kinetics of these channels.

1. INTRODUCTION

In the first paper of this series we proposed (1) a model which included cooperativity between the presumably protein subunits of a potassium channel in the squid giant axon membrane. Subsequent calculations designed to test this proposal (2, 3) indicated, however, that at most there could be only a small amount of cooperativity. We therefore omit cooperativity in the present paper, just as Hodgkin and Huxley (4) did implicitly in their formalism.

The power density spectrum $G(f)$ of fluctuations in the steady-state axon membrane current (5, 6) is found experimentally to have the form $1/f$ at least over the range $f = 20$ to several thousand hertz (cycles per second). Furthermore, these fluctuations are associated with the potassium current (5, 6). The theoretical basis of these observations is, however, not yet known.

Potassium channels open and close with millisecond kinetics (4); $1/\tau = O$ (10^3 Hz). On the other hand, the time constant τ' for the kinetic processes involved in transport of K^+ through an already open channel is of order of 10 μsec, from the establishment of the "instantaneous current" (7); $1/\tau' = O$ (10^5 Hz).

This separation into two time domains lies at the heart of the HH formulation. Offhand, it would seem reasonable to expect, then, that a theoretical calculation of $G(f)$ from the HH model for the open-close kinetics of K^+ channels might give the experimental $G \sim 1/f$ in the 10^3 Hz range. That this is not the case is apparently taken for granted by experts in the field. We feel, however, that an explicit demonstration is called for, especially one which also considers various modifications of HH which could conceivably lead to $1/f$ behavior. Incidentally, these modifications may be of some interest quite aside from the noise problem. Our negative results (below) based on open-close kinetics are consistent with the generally held view (5) that the observed $G \sim 1/f$ is associated primarily with K^+ current through open K^+ channels.

2. NOISE FROM FLUCTUATIONS IN THE NUMBER OF OPEN HH K^+ CHANNELS

For simplicity we give here the derivation of $G(f)$ only for the case $x = 2$. The result for arbitrary x is derived in Appendix I. We have also derived $G(f)$ in the $x = \infty$ case (8) and obtained the same result by letting $x \to \infty$ in the formula for arbitrary x.

We consider an ensemble of M independent HH K^+ channels with $x = 2$. The kinetic scheme (2) is

$$\begin{array}{ccccc} [0] & \underset{\beta}{\overset{2\alpha}{\rightleftarrows}} & [1] & \underset{2\beta}{\overset{\alpha}{\rightleftarrows}} & [2] \\ (p_0) & & (p_1) & & (p_2) \end{array} \qquad (1)$$

The mean number of open channels at equilibrium is

$$\bar{N}_2^e = Mp_2^e = Mn_\infty^2 = M\left(\frac{\alpha}{\alpha+\beta}\right)^2. \qquad (2)$$

The mean steady-state K^+ current is

$$\bar{I}_K = g_K \bar{N}_2^e (V - E_K). \qquad (3)$$

We assume here, as indicated above, that fluctuations in I_K on a millisecond time scale arise from fluctuations in N_2^e, the number of open channels at equilibrium. Fluctuations in $g_K(V - E_K)$, the steady-state current through a single open channel, occur (we assume) on a 10 μsec time scale. We therefore treat the latter, in the millisecond region, as a constant averaged quantity. Thus we can begin by deriving the power spectrum of fluctuations in N_2^e, and insert the constant factor $g_K(V - E_K)$ at the end of the derivation.

The fundamental equation we use, the Wiener-Khintchine theorem (9), is

$$G(f) = 4\int_0^\infty C(t)\cos 2\pi f t \, dt, \qquad (4)$$

where $C(t)$, the correlation function, is an ensemble average:

$$C(t) = \langle [N_2(0) - \bar{N}_2^e] \cdot [N_2(t) - \bar{N}_2^e] \rangle. \tag{5}$$

To be more explicit: given arbitrary values, i.e. an arbitrary fluctuation, $N_1(0)$ and $N_2(0)$ at $t = 0$ [$N_0(0)$ is not independent], we first find the consequent mean value of $N_2(t) - \bar{N}_2^e$ (regression of the fluctuation). This can be done using the two first-order differential equations in the mean values $\bar{N}_1$ and $\bar{N}_2$, which follow from equation 1; i.e., more detailed stochastic treatment of equation 1 is not necessary. In solving the differential equations, the initial values of $\bar{N}_1$ and $\bar{N}_2$ are $N_1(0)$ and $N_2(0)$. The remaining step is to average the product

$$[N_2(0) - \bar{N}_2^e] \cdot [\bar{N}_2(t; N_1(0), N_2(0)) - \bar{N}_2^e], \tag{6}$$

over the equilibrium probability distribution in $N_1(0)$ and $N_2(0)$.

At equilibrium, the probability that the ensemble has N_1 channels in state 1 and N_2 in state 2 is

$$P^e_{N_1 N_2} = \frac{M!(p_0^e)^{M-N_1-N_2}(p_1^e)^{N_1}(p_2^e)^{N_2}}{N_1!N_2!\,(M - N_1 - N_2)!}, \tag{7}$$

where

$$p_0^e = (1 - n_\infty)^2, \qquad p_1^e = 2n_\infty(1 - n_\infty), \qquad p_2^e = n_\infty^2. \tag{8}$$

It follows from equations 7 and 8 that (10)

$$\sigma^2_{N_2} \equiv \langle (N_2 - \bar{N}_2^e)^2 \rangle = Mp_2^e(1 - p_2^e) = Mn_\infty^2(1 - n_\infty^2), \tag{9}$$

and

$$\sigma^2_{N_1 N_2} \equiv \langle (N_1 - \bar{N}_1^e)(N_2 - \bar{N}_2^e) \rangle = -Mp_1^e p_2^e = -2Mn_\infty^3(1 - n_\infty). \tag{10}$$

The result of the first step mentioned above is

$$\bar{N}_2(t) - \bar{N}_2^e = \{2n_\infty[N_2(0) - \bar{N}_2^e] + n_\infty[N_1(0) - \bar{N}_1^e]\}e^{-t/\tau} + \{(1 - 2n_\infty)[N_2(0) - \bar{N}_2^e] - n_\infty[N_1(0) - \bar{N}_1^e]\}e^{-2t/\tau}, \tag{11}$$

where $\tau = 1/(\alpha + \beta)$. When we substitute equation 11 in equation 6 and average over the equilibrium distribution in $N_1(0)$ and $N_2(0)$, we simply encounter equations 9 and 10 in the averaging process. Thus we find

$$C(t) = Mn_\infty^2[2n_\infty(1 - n_\infty)e^{-t/\tau} + (1 - n_\infty)^2 e^{-2t/\tau}]. \tag{12}$$

This reduces to equation 9 when $t = 0$, as it should. Finally, from equation 4,

$$G(\omega) = 8M\tau n_\infty^2(1 - n_\infty)\left(\frac{n_\infty}{1 + \omega^2\tau^2} + \frac{1 - n_\infty}{4 + \omega^2\tau^2}\right), \quad (13)$$

where $\omega = 2\pi f$. The total fluctuation (all frequencies) in N_2^e is $\int_0^\infty G(f)\,df$. This operation also leads to equation 9, as expected.

For arbitrary x (see Appendix I)

$$C(t) = Mn_\infty^x\{[n_\infty + (1 - n_\infty)e^{-t/\tau}]^x - n_\infty^x\}, \quad (14)$$

and

$$G(\omega) = 4Mx\tau n_\infty^x(1 - n_\infty)\sum_{i=1}^{x}\frac{(x-1)!n_\infty^{x-i}(1 - n_\infty)^{i-1}}{(i-1)!(x-i)!(i^2 + \omega^2\tau^2)}; \quad (15)$$

In the limit $x \to \infty$ (8), equations 14 and 15 become

$$C(t) = Me^{-2\mu_\infty}[\exp(\mu_\infty e^{-t/\tau}) - 1], \quad (16)$$

and

$$G(\omega) = 4M\tau e^{-2\mu_\infty}\sum_{i=1}^{\infty}\frac{\mu_\infty^i}{(i-1)!(i^2 + \omega^2\tau^2)}, \quad (17)$$

where $e^{-\mu_\infty}$ is the fraction of open channels (8). In order to derive equation 16 directly (which we have done), one needs to use equation 32 of Shimoda et al. (11), which is the appropriate solution of the infinite set of first-order differential equations.

To convert equations 15 and 17 into power spectra $G_I(\omega)$ in the K^+ current (see equation 3), we use

$$G_I(\omega) = G(\omega)\cdot g_K^2(V - E_K)^2. \quad (18)$$

Fig. 1 shows a plot of $\log_{10} G_I'$ vs. $\log_{10}\omega$, for several values of V, in the HH axon 17 case (Appendix II) $x = 4$, $n_0 = 0.15$. G_I' is defined for convenience (for any x) by

$$G_I = 16Mg_K^2G_I'. \quad (19)$$

τ is in milliseconds, and $\omega = 2\pi f$ (milliseconds)$^{-1}$. The lower abscissa scale is f in hertz (seconds^{-1}). The two straight lines show slopes of -1 ($G \sim 1/f$) and -2 ($G \sim 1/f^2$). The calculated $G(f)$ is constant at low frequencies and goes as $1/f^2$ at higher frequencies. The experimental $G \sim 1/f$ behavior between about $\log_{10}\omega = -1$ and $+1$ is not found. The theoretical dependence on V is also rather unlike what is observed (5).

We have calculated the same family of curves for the cases (Appendix II) $x = 10$,

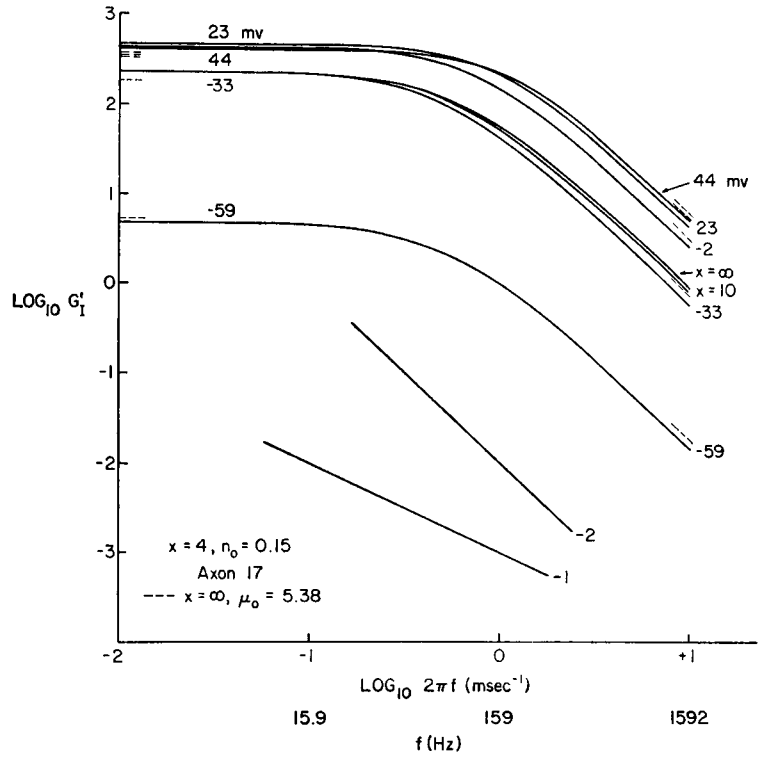

FIGURE 1 Noise power spectrum based on HH open-close K^+ channel kinetics. Axon 17, $x = 4$, $n_0 = 0.15$. The dashes show the beginnings and ends of the $x = \infty$, $\mu_0 = 5.38$ family of curves. Curves labeled $x = 10$ ($n_0 = 0.54$) and $x = \infty$ ($\mu_0 = 5.38$) are for $V = -33$ mv (see text). The straight line labeled -1 shows the slope found experimentally (vertical position arbitrary) between about $\log_{10} 2\pi f = -1$ and $+1$.

$n_0 = 0.54$ and $x = \infty$, $\mu_0 = 5.38$. The general pattern is identical with $x = 4$ so we omit additional figures, but we include $V = -33$ mv curves for $x = 10$ and $x = \infty$ in Fig. 1. A slight vertical adjustment was used for $x = 10$ and ∞ to make the three curves start off together. In addition, the dashes show the beginnings and ends of the $x = \infty$ curves without adjustment. Clearly, variations in x do not account for the discrepancy between theory and experiment referred to above.

3. NOISE AND VARIATIONS ON HODGKIN-HUXLEY

For completeness, we have also tried $G(\omega)$ calculations for three modifications of the HH model. None of these is helpful. Details in the first two cases are relegated to appendices.

Conduction by "Closed" Channels

Here, as explained in Appendix III, we assume that a channel with one or more subunits in the "wrong" conformation has a reduced conductance but is not absolutely

closed to K^+. We find in this case that equations 14 and 15 are generalized to

$$C(t) = MN_\infty^x\{[N_\infty + Ae^{-t/\tau}]^x - [n_\infty + \kappa(1 - n_\infty)e^{-t/\tau}]^x\}, \qquad (20)$$

and

$$G(\omega) = 4Mx\tau N_\infty^x A \sum_{i=1}^{x} \frac{(x-1)!N_\infty^{-i}A^{i-1}}{(i-1)!(x-i)!(i^2 + \omega^2\tau^2)}, \qquad (21)$$

where

$$A = n_\infty(1 - \kappa)(1 - N_\infty)/N_\infty. \qquad (22)$$

We have calculated $G(\omega)$ for $x = 4$ and various values of κ with $V = 50$ and -50 mv using HH values for N_∞ and τ. The curves have essentially the same shape as those in Fig. 1.

Nonidentical Subunits

In this modification (details in Appendix IV), it is assumed that the x subunits of a K^+ channel may have somewhat different properties. It is easy to see, then, that when, for example, $x = 4$,

$$G(\omega) = 4Mn_1n_2n_3n_4\left[\frac{n_1n_2n_3(1-n_4)\tau_4}{1+\omega^2\tau_4^2} + 3 \text{ terms} + \frac{n_1n_2(1-n_3)(1-n_4)\bar{\tau}_{34}}{1+\omega^2\bar{\tau}_{34}^2} + 5 \text{ terms} + \cdots + \frac{(1-n_1)(1-n_2)(1-n_3)(1-n_4)\bar{\tau}_{1234}}{1+\omega^2\bar{\tau}_{1234}^2}\right], \qquad (23)$$

where the total numbers of terms of each type are 4, 6, 4, 1, respectively (binomial coefficients), and where

$$\bar{\tau}_{34} \equiv \frac{\tau_3\tau_4}{\tau_3 + \tau_4}, \qquad \bar{\tau}_{234} \equiv \frac{\tau_2\tau_3\tau_4}{\tau_2\tau_3 + \tau_2\tau_4 + \tau_3\tau_4}, \quad \text{etc.} \qquad (24)$$

The subscript ∞ has been dropped from the n_i's in equation 23.

We have calculated $G(\omega)$ for the four cases shown in equations 50 and 51, with $V = +50$ mv and $V = -50$ mv. Again the curves have the same shape as those in Fig. 1.

Incidentally, equation 23 gives the steady-state $G(\omega)$ for an HH Na^+ channel. That is, we simply put

$$n_1 = n_2 = n_3 = m_\infty, \qquad \tau_1 = \tau_2 = \tau_3 = \tau_m, \qquad n_4 = h_\infty, \qquad \tau_4 = \tau_h, \qquad (25)$$

and combine like terms.

Nonidentical Channels

Consider the hypothetical situation in which there is a distribution of HH τ values among the K^+ channels. We do not make this suggestion because we believe in it but because it is well known in semiconductor physics that a sufficiently broad distribution $W(\tau)$ in τ values of the form $1/\tau$, corresponding to a constant or flat distribution in activation energies for the τ process, will give $G(f) \sim 1/f$. It is conceivable, for example, that the bilayer environment around K^+ channels is variable and thus produces a distribution in channel properties.

It is rather obvious, however, that superposition will be lost in a generalization of this sort, but let us try out this idea in the simple case in which there is a distribution in τ values but not in n_∞ values; i.e., the activation energy barrier between subunit conformations *i* and *ii* varies, but not the equilibrium free energy difference.

We write the normalized distribution function as

$$W(\tau) = 1/2\,\tau \ln a \qquad (a \geq 1) \tag{26}$$

between $\tau_1 = \tau_0/a$ and $\tau_2 = a\tau_0$; otherwise $W(\tau) = 0$. The larger a, the broader the distribution. The HH case is $a = 1$. Denote, now, the right-hand side of equation 15 when $x = 4$ by $G(\omega, \tau)$. Then, in the present model

$$G(\omega) = \int_{\tau_1}^{\tau_2} G(\omega, \tau) W(\tau)\, d\tau. \tag{27}$$

We have calculated $G(\omega)$ from equation 27 for $x = 4$ using HH $n_\infty(V)$ and $\tau_0(V)$ values for $V = +50$ and -50 mv. We found that, when the results are plotted as in Fig. 1 where $a = 1$, even the $a = 10$ curves have considerable curvature between $\log_{10} 2\pi f = -1$ and $+1$ while the $a = 100$ curves probably barely qualify as having the required behavior (approximate straight line with slope -1) in the same range. That is, it takes $a \geq 100$ to produce $G \sim 1/f$.

We made the usual (2, 3) kind of superposition check for this model ($x = 4$), with depolarizations to $V = +50$ mv, using for the fraction of open channels

$$p_K(t) = \int_{\tau_1}^{\tau_2} n^4(t, \tau) W(\tau)\, d\tau. \tag{28}$$

Superposition is perfect for $a = 1$ but deteriorates as a increases; it is already very bad for $a = 2$. Also, initial induction behavior becomes unsatisfactory between $a = 2$ and $a = 10$. The obvious conclusion is that this kind of modification of HH is not an acceptable way to produce $G(f) \sim 1/f$.

APPENDIX I

We generalize here the derivation of $C(t)$ given in section 2 from $x = 2$ to arbitrary x. The kinetic scheme is now

$$[0] \underset{\beta}{\overset{x\alpha}{\rightleftarrows}} [1] \underset{2\beta}{\overset{(x-1)\alpha}{\rightleftarrows}} \cdots \underset{x\beta}{\overset{\alpha}{\rightleftarrows}} [x] \quad . \tag{29}$$
$$(p_0) \qquad (p_1) \qquad\qquad\qquad (p_x)$$

The probability that a channel is open is p_x . At equilibrium,

$$p_j^e = \frac{\bar{N}_j^e}{M} = \frac{x!\, n_\infty^j (1 - n_\infty)^{x-j}}{j!(x-j)!}, \tag{30}$$

and (10)

$$\begin{aligned} \sigma_{ij}^2 &= -Mp_i^e p_j^e \qquad (i \neq j) \\ &= Mp_i^e(1 - p_i^e) \qquad (i = j), \end{aligned} \tag{31}$$

where

$$n_\infty = \alpha/(\alpha + \beta). \tag{32}$$

The solution of the set of linear differential equations in the $p_j(t)$, corresponding to the kinetic scheme, equation 29, can be expressed formally as

$$p(t) = \Phi(t)p^0, \tag{33}$$

where $\Phi(t)$ is the fundamental matrix of the system and p^0 is the column matrix of initial probabilities ($t = 0$). $\Phi(t)$ depends on the quantities which appear in equation 29 but is independent of the initial state.

The only properties of $\Phi(t)$ which we shall need follow from a special case. Suppose the ensemble of channels is at equilibrium at $t = 0$ with n_0 in place of n_∞ in equation 30. This equation gives the p_k^0 for use in equation 33 in this case. A binomial distribution is then maintained at all t (2, 12):

$$p_j(t) = \frac{x!\, n(t)^j [1 - n(t)]^{x-j}}{j!(x-j)!} = \sum_{k=0}^{x} \phi_{jk}(t) p_k^0, \tag{34}$$

where

$$n(t) = n_\infty + (n_0 - n_\infty)e^{-t/\tau}. \tag{35}$$

If we now choose, first, $n_0 = 1$ and then $n_0 = n_\infty$, we obtain from equation 34 the required properties of $\Phi(t)$:

$$\phi_{xx}(t) = [n_\infty + (1 - n_\infty)e^{-t/\tau}]^x \qquad (\text{from } n_0 = 1), \tag{36}$$

$$p_x(t) \equiv n_\infty^x = \sum_{k=0}^{x} \phi_{xk}(t) p_k^e \qquad (\text{from } n_0 = n_\infty). \tag{37}$$

Let $\bar{N}_x(t; \{N_i(0)\})$ represent the value of $\bar{N}_x \equiv Mp_x$ at t, having started from the arbitrary state $\{N_i(0)\} \equiv N_1(0), N_2(0), \cdots, N_x(0)$ at $t = 0$. Then

$$C(t) = \langle [N_x(0) - \bar{N}_x^e] \cdot [\bar{N}_x(t; \{N_i(0)\}) - \bar{N}_x^e] \rangle, \tag{38}$$

where the average is over the equilibrium probability distribution in the $N_i(0)$ (see equation 7).

According to equation 33,

$$\bar{N}_x(t; \{N_i(0)\}) = \sum_{k=0}^{x} \phi_{xk}(t) N_k(0). \tag{39}$$

If we now substitute equation 39 into equation 38, we find

$$C(t) = \sum_{k=0}^{x} \phi_{xk}(t) \langle N_x(0) N_k(0) - \bar{N}_x^e \bar{N}_k^e \rangle, \tag{40}$$

$$= \sum_{k=0}^{x} \phi_{xk}(t) \sigma_{xk}^2, \tag{41}$$

$$= -Mp_k^e \sum_{k=0}^{x} \phi_{xk}(t) p_k^e + M\phi_{xx}(t) p_x^e, \tag{42}$$

$$= Mn_\infty^x \{[n_\infty + (1 - n_\infty) e^{-t/\tau}]^x - n_\infty^x\}, \tag{43}$$

where we have used equations 30, 31, 36, and 37.

APPENDIX II

Hodgkin and Huxley (4) present a family of 12 potassium conductance curves for their axon 17 depolarizations from the rest potential, r.p. The correspondence between the theoretical curves and the experimental points is fairly good, but the theoretical curves do fail to reproduce satisfactorily the rather pronounced induction behavior of the experimental points near $t = 0$. It seems to us that this discrepancy might be due simply to a choice by HH of n_∞ at r.p. (denoted by n_0 below) which is too large; relatively exotic features such as cooperativity (1–3) need not be invoked. This is not meant to be much more than a suggestion for future evaluation, but we do use it here.

Our analysis (see below) for $x = 4$ suggests that n_0 might be in the neighborhood of 0.15 rather than $n_0 = 0.32$ (HH). This would reduce g_{K0} for axon 17 from the already small value 0.24 mmho cm^{-2} (HH) to about 0.01 mmho cm^{-2}. To account for the potassium current at r.p. thereby "lost," we presume that there is some additional K^+ transport (with g_K') through the membrane, other than via specialized (HH) K^+ channels and not already included in the HH leakage. (One could further assume, as seems to be required [7], that $g_K' \to 0$ at strongly hyperpolarizing potentials.) Hence we use, for a "corrected" channel g_K,

$$g_K \cong g_K(\text{HH}) - 0.24.$$

For $x = 4$, we estimated experimental slopes, from the whole family of axon 17 curves, at $t_{1/2}$ (where $g_K = g_{K\infty}/2$) and used these to compute τ values. These τ values, in turn, allowed calculation of n_0 values from the experimental $t_{1/2}$ values. The n_0 values ranged between 0.06 and 0.25; we settled on $n_0 = 0.15$ as a representative value for the whole set (the HH model requires that a single n_0 be used). Finally, we employed $n_0 = 0.15$ and the $t_{1/2}$ values to calculate a revised set of τ values. These are presented in Table I. The computed $g_K(t)$ curves, based on Table I, give an extremely good fit of the HH experimental points, including the early induction behavior.

TABLE I

POTASSIUM CONDUCTANCE TIME CONSTANTS FOR AXON 17

ΔV	$t_{1/2}$	τ
mv	*msec*	*msec*
109	1.530	0.917
100	1.594	0.956
88	1.819	1.094
76	2.112	1.273
63	2.407	1.456
51	2.87	1.744
38	2.63	2.225
32	4.20	2.59
26	4.76	2.96
19	5.65	3.57
10	5.72	3.92
6	5.49	3.94

Axon 17, $x = 4$, $n_0 = 0.15$.

A similar procedure was followed for $x = 3$, 6, 8, 10, and ∞, using in every case the experimental $t_{1/2}$ values in Table I. The respective values of n_0 selected were 0.08, 0.31, 0.45, 0.54, and $\mu_0 = 5.38$ (for $x = \infty$; see reference 8). A different set of τ values was obtained for each value of x.

The fit of the HH points by computed curves $g_K(t)$ for $x = 3$, 6, and 8 was also extremely good and practically indistinguishable from $x = 4$. The fit for $x = 10$ and $x = \infty$, at the larger values of ΔV (Table I), was noticeably less satisfactory. For $x = 2$, the early induction was clearly unsatisfactory even with $n_0 = 0$, which is the most favorable case for this property.

It would appear that depolarization kinetic data of this sort cannot be used alone to distinguish between $x = 3$ to about $x = 8$. There is certainly no counterindication here to the HH choice of $x = 4$. If the channel or gate is indeed a protein complex of x subunits, $x = 4$ is an especially attractive number (13). Incidentally, we do not interpret Fig. 2 of Cole and Moore (14) as favoring $x = 6$ over $x = 4$, since $\tau_{x=6}$ was used for the $x = 4$ curve. Essentially the same fit of the data could have been made with $x = 4$, which is what we find here with the HH data, where we use a different τ set for each x.

APPENDIX III

Conduction by Closed Channels

It is somewhat unreasonable, physically, to assume that a K^+ channel is "open" only if *all* subunits are in state *ii* (2, 4). Let us suppose instead (8) that if one subunit of the complex (channel or gate) is in state *i*, there is an additional steric or electrostatic energy barrier W to passage of a K^+ ion, with the result that the conductance of an all-*ii* channel is reduced by a factor $\kappa = e^{-W/kT} < 1$ (HH use, implicitly, $\kappa = 0$). If two subunits are in state *i*, the simplest and most likely assumption is that the barrier is $2W$ and hence that the conductance reduction factor is κ^2, etc. (If the barriers W are consecutive, i.e. in series, rather than "simultaneous," a simple argument suggests that we could then use κ, $\kappa/2$, $\kappa/3$, etc, but we shall not

consider this case further here.) W and κ may (electrostatic, above) or may not (steric) be V-dependent (as are α and β).

Thus the HH relation $g_K = \bar{g}_K n^x$ is generalized to

$$g_K = g_K\left[n^x + xn^{x-1}(1-n)\kappa + \frac{x(x-1)}{2}n^{x-2}(1-n)^2\kappa^2 + \cdots\right],$$

$$= g_K[n + \kappa(1-n)]^x = g_K N^x, \tag{44}$$

where

$$N = n + \kappa(1-n), \tag{45}$$

$$0 \leq n \leq 1, \qquad \kappa \leq N \leq 1. \tag{46}$$

It is then easy to show that

$$N(t) = N_\infty + (N_0 - N_\infty)e^{-t/\tau}, \tag{47}$$

where $\tau = 1/(\alpha + \beta)$ as usual. If κ is a function of V, the κ in N_0 is κ_0 and the κ in N_∞ is κ_∞ (of course in τ, $\alpha \equiv \alpha_\infty$ and $\beta \equiv \beta_\infty$). Equations 44 and 47 are formally the same as in HH. Hence $g_K(t)$ curves cannot be used to distinguish between the two models, i.e., to detect a $\kappa > 0$.

The induction behavior would depend here on N_0 just as on n_0 in Appendix II; but $N_0 \geq \kappa$. Thus, for example, if $x = 4$, our analysis in Appendix II suggests that $\kappa \leq 0.15$, i.e. we could have $n_0 = 0$ and $\kappa = 0.15$ or, at the other extreme, $n_0 = 0.15$ and $\kappa = 0$ as in Appendix II.

The deduction from τ and N_∞ (obtained by experimental curve fitting) of separate values of α and β depends on the value of κ since

$$\alpha = \frac{N_\infty - \kappa}{\tau(1-\kappa)} \quad \text{and} \quad \beta = \frac{1 - N_\infty}{\tau(1-\kappa)}. \tag{48}$$

Also, the subunit equilibrium constant for $i \rightleftarrows ii$ is

$$\frac{n_\infty}{1-n_\infty} = \frac{\alpha}{\beta} = \frac{N_\infty - \kappa}{1 - N_\infty}. \tag{49}$$

If indeed $\kappa > 0$, some but not all of the HH analysis would have to be revised, but there seems to be no information available concerning κ at the present time.

APPENDIX IV

Nonidentical Subunits

We have been assuming implicitly, with HH, that all of the x subunits in a K^+ channel have the same properties, e.g., $\alpha(V)$ and $\beta(V)$. There are two obvious ways in which this assumption could break down:

(*a*) The subunits are identical but are sufficiently separated spatially so that they "see" different effective local potentials. Of course if α and β depend on the local electric field and not on the local potential, and if the field is essentially constant, as is often assumed, over the space occupied by the subunits, then no effect of the type under consideration here would be observed; i.e., this latter situation would be indistinguishable from HH.

(*b*) The subunits are actually different and have different $\alpha(V)$'s and $\beta(V)$'s. They might also be spatially separated.

In this more general model, the fraction of K^+ channels open (p_K) would be $n_1 n_2 \cdots n_x$ rather than n^x. It is easy to see, however, that Cole-Moore superposition (2, 3, 14), as found experimentally, would not be a property of channels of this type.

To determine the extent of failure of superposition (2, 3) that might be expected, we made computations on two examples, designed to illustrate cases *a* and *b* above, respectively. Let $\alpha(V)$ and $\beta(V)$ be the HH composite functions (4). In these two examples ($x = 4$), we assume for convenience that the HH functions apply to all subunits but, for a membrane potential V, we use $\alpha(V_i)$ and $\beta(V_i)$ for the ith subunit, where:

$$V_1 = 0.70V, \quad V_2 = 0.90V, \quad V_3 = 1.10V, \quad V_4 = 1.30V, \tag{50 a}$$

$$V_1 = V - 30, \quad V_2 = V - 10, \quad V_3 = V + 10, \quad V_4 = V + 30. \tag{50 b}$$

The V_i are effective membrane, not local, potentials. We used various conditioning potentials V(2, 3) and a final value $V = +50$ mv. In both cases, the quality of superposition is not very good (beyond experimental error). However, in the less extreme cases,

$$V_1 = 0.85V, \quad V_2 = 0.95V, \quad V_3 = 1.05V, \quad V_4 = 1.15V, \tag{51 a}$$

$$V_1 = V - 15, \quad V_2 = V - 5, \quad V_3 = V + 5, \quad V_4 = V + 15, \tag{51 b}$$

the superposition is almost perfect. Thus a small amount of variability in the properties of the subunits cannot be excluded by this test. The same was found to be true of a small amount of cooperativity (2, 3).

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