FREQUENCIES OF PAIRED OPEN–CLOSED DURATIONS OF ION CHANNELS

Method of Evaluation From Single-Channel Recordings

IZCHAK Z. STEINBERG
Chemical Physics Department, Weizmann Institute of Science, Rehovot, Israel 76100

ABSTRACT The probability of the occurrence of consecutive closed–open or open–closed intervals of specified durations in single-channel recordings may be of enormous help in the establishment of the kinetic scheme that describes the behavior of the channel. The relevant probability functions are linear combinations of products of exponential functions of the closed durations and the open durations. A method is presented for the evaluation of the coefficients of the exponential functions using a set of auxiliary functions that are each orthogonal to all but one of the exponential functions. The coefficients in the probability functions may then be obtained from the experimental data by multiplication by the auxiliary functions and subsequent simple integration operations. Furthermore, the variance to be expected in the evaluated numerical magnitude of the parameters, due to the stochastic nature of the transitions in the channel conductance, is also readily estimated by use of the above auxiliary functions. The procedure is illustrated by analysis of synthetic data obtained from computer simulated experiments.

INTRODUCTION

Recordings of the conductance of a single ion channel usually fluctuate between a zero conductance level and a single, finite conductance amplitude. The channel may, however, be in a few molecular states in each of these conductance levels. For example, sodium and potassium voltage-sensitive channels of the Hodgkin and Huxley type may be in seven and five different nonconducting states, respectively (Hodgkin and Huxley, 1952). The transitions of a channel from one state to another are customarily assumed to be Markov processes (Colquhoun and Hawkes, 1977; 1981), i.e., the probabilities of interconversion at any given moment depend only on the state in which the channel is at that moment, and not on the history past to that moment. Thus the durations of the dwell times in any given state should be monoexponentially distributed. However, if there is more than one state that has a given conductance level, the durations observed for the channel at this conductance amplitude in a single-channel recording are expected to be distributed multiexponentially. Furthermore, if the durations of both conductance levels are distributed multiexponentially, the probability of observing the channel to be open for a given specified duration does generally depend on the closed time duration that has occurred before, and vice versa (Fredkin et al., 1985; Steinberg, 1986). This interdependence of durations of consecutive open and closed intervals is closely related to the kinetic scheme of interconversions among the various molecular states in which the channel can be, and hence the interest in the study of the joint probabilities of the occurrence of closed–open and open–closed sequences of specified durations. It has recently been shown that these joint probabilities can also be used to obtain information as to whether the interconversions among the various states of the ion channel obey detailed balance, i.e., whether or not the channel behaves like a system at thermodynamic equilibrium (Steinberg, 1986). Thus, if all interconversions among states obey detailed balance, the joint probability–density distribution function $p(t_c, t_o)$ for the occurrence of a time interval t_c, during which the channel is not conducting, followed by a time interval t_o, during which the channel is conducting, must be equal to the probability–density distribution function $p^r(t_c, t_o)$ for the occurrence of the time intervals in the reverse order. This might be useful for revealing whether irreversible processes are coupled to the gating of the ion channels. In view of the extensive information that may be derived from these joint probability–density distribution functions about the dynamic processes that occur in ion channels, it might be advantageous to have a convenient and reliable method for the evaluation

[1]It can be readily shown that in a hypothetical case in which the events in a single-channel recording cluster into groups of two fully correlated events each, the statistical variance in the calculated values of the parameters a_{ij} is twice the magnitude of the variance in the case in which the events are completely uncorrelated. The root-mean-square deviations of a_{ij} will differ by a factor of $\sqrt{2}$ in the two cases. In practice the difference between the variance in the evaluated parameters and the variance obtained for a series of uncorrelated events is expected to be significantly smaller than that in the above hypothetical case.

of these probability functions from single-channel recordings.

The joint probability–density distribution functions $p(t_c, t_o)$ and $p^r(t_c, t_o)$ are linear combinations of products of exponential decay functions of t_c and t_o (Fredkin et al., 1985; Steinberg, 1986). The lifetimes appearing as parameters in these functions are identical to those that appear in the probability–density distribution functions, $p_c(t_c)$ and $p_o(t_o)$, for the durations of closed intervals and open intervals, respectively, in the single-channel recording under study. Methods are available for the evaluation of these lifetimes from single-channel recordings (Colquhoun and Sigworth, 1983; Sigworth, 1983; see also Grinvald and Steinberg, 1974). Henceforth it will be assumed that the numerical values of the lifetimes have been obtained by use of such procedures. We are thus left with the task of determining the coefficients of the products of the exponential functions that appear in the expressions for $p(t_c, t_o)$ and $p^r(t_c, t_o)$, given the data collected by single-channel recording techniques. In addition, if the values obtained for the coefficients are to be significant, it is essential to have an estimate of the range of error that is likely to occur in the estimated parameters. A convenient approach is presented below for achieving these goals.

METHOD OF ANALYSIS

Let us denote by τ_i and θ_j the various lifetimes in the multiexponential functions that represent the probability–density distribution functions $p_c(t_c)$ and $p_o(t_o)$ for the durations of the nonconducting intervals t_c and the conducting intervals t_o, respectively, in a single-channel recording. The integer indices i and j vary from 1 to I and from 1 to J, respectively, where I and J are the total number of exponentials in the respective function $p_c(t_c)$ and $p_o(t_o)$. Thus,

$$p_c(t_c) = \sum_{i=1}^{I} \alpha_i f_i$$

$$p_o(t_o) = \sum_{j=1}^{J} \beta_j g_j, \tag{1}$$

where α_i and β_j are constants and f_i and g_i are defined as,

$$f_i = (1/\tau_i) \exp(-t_c/\tau_i)$$

$$g_j = (1/\theta_j) \exp(-t_o/\theta_j). \tag{2}$$

The integral of each of the functions f_i and g_j over the time range 0 to ∞ is unity. A parameter α_i thus denotes the probability that a closed interval in the single-channel recording belongs to the linear combination of closed states (i.e., the "component" of the closed states; see Steinberg, 1986), which decays with the lifetime τ_i. Similarly, a parameter β_j denotes the probabilities that an open interval in the single-channel recording belongs to the linear combination of open states (i.e., the "component" of the open states), which decays with the lifetime θ_j.

The joint probability–density distribution function $p(t_c, t_o)$ can then be represented by a linear combination of pair products of exponential functions of t_c and t_o with lifetimes τ_i and θ_j, respectively (Fredkin et al., 1985; Steinberg, 1986). Thus,

$$p(t_c, t_o) = \sum_{i=1}^{I} \sum_{j=1}^{J} a_{ij} f_{ij}, \tag{3}$$

where the coefficients a_{ij} are constants and the functions f_{ij} are defined as,

$$f_{ij} = (1/\tau_i) \exp(-t_c/\tau_i) \cdot (1/\theta_j) \exp(-t_o/\theta_j). \tag{4}$$

Obviously, $p^r(t_c, t_o)$ can be represented similarly and will not be treated separately. Note that integration of each of the functions f_{ij} over the whole ranges of positive t_c and t_o yields the value of unity. So does integration of $p(t_c, t_o)$. It thus follows that,

$$\sum_{i=1}^{I} \sum_{j=1}^{J} a_{ij} = 1 \tag{5}$$

and each a_{ij} has the significance of representing the fractional volume under the function $p(t_c, t_o)$, i.e., the relative number of pairs of closed–open events in the single-channel recording, which is contributed by the corresponding function f_{ij}. Each of the coefficients a_{ij} also expresses the relative frequency of the occurrence of a transition from the component in $p_c(t_c)$ which decays with the lifetime τ_i to the component in $p_o(t_o)$ which decays with the lifetime θ_j (Steinberg, 1986). The coefficients a_{ij} are thus related to the correlations in the durations of consecutive closed and open intervals. For example, if no correlations of this kind exist, $a_{ij} = \alpha_i\beta_j$.

As stated above, it will be assumed that the numerical values of the various lifetimes τ_i and θ_j are known by analysis of $p_c(t_c)$ and $p_o(t_o)$, respectively. To obtain the mathematical expression for $p(t_c, t_o)$ one additionally needs to evaluate the numerical values of the various coefficients a_{ij}. To this end, let us define a set of auxiliary functions $F^{\alpha\beta}$ as follows:

$$F^{\alpha\beta} = \sum_{i=1}^{I} \sum_{j=1}^{J} b_{ij}^{\alpha\beta} f_{ij}, \tag{6}$$

where α and β are integer indices varying from 1 to I and from 1 to J, respectively. The number of functions $F^{\alpha\beta}$ is thus $I \cdot J$. The coefficients $b_{ij}^{\alpha\beta}$ are constants, the values of which are prescribed by the requirement that the functions $F^{\alpha\beta}$ fulfill the following set of relationships,

$$\int_0^\infty \int_0^\infty F^{\alpha\beta} f_{ij}\, dt_c dt_o = \delta_i^\alpha \cdot \delta_j^\beta$$
$$i = 1, 2, \ldots, I$$
$$j = 1, 2, \ldots, J$$
$$\alpha = 1, 2, \ldots, I$$
$$\beta = 1, 2, \ldots, J, \tag{7}$$

where δ_i^α and δ_j^β are each a Kronecker delta, which assumes a value of 1 if the numerical values of the indices are the same and assumes a value of zero otherwise. Each function $F^{\alpha\beta}$ is thus orthogonal to all of the f_{ij} functions except the one for which $i = \alpha$ and $j = \beta$. For this specific function, f_{ij}, the integral in Eq. 7 is equal to unity. Note that Eq. 7 represents a large number of equations. Specifically, for every given value of α and value of β there are $I \cdot J$ equations that have to be satisfied, corresponding to the various combinations of the values that i and j can assume. Thus, for every function $F^{\alpha\beta}$, which has $I \cdot J$ as yet unknown parameters $b_{ij}^{\alpha\beta}$ there are $I \cdot J$ simultaneous equations that have to be obeyed. These simultaneous equations determine the values that the parameters $b_{ij}^{\alpha\beta}$ must assume. Note that the integrands in Eq. 7 are exponential functions, the integration of which may be performed analytically. A specific example for the case in which the closed durations and the open durations are resolved into two exponentials each (i.e., $I = J = 2$) is worked out in some detail below and may serve to illustrate the practical details of the calculations.

The properties of the functions $F^{\alpha\beta}$ expressed by Eq. 7 make these functions a very convenient tool for the straightforward evaluation of the unknown parameters a_{ij} in a given function $p(t_c, t_o)$, which is presented numerically or graphically, by use of the following procedure:

$$\int_0^\infty \int_0^\infty p(t_c, t_o)\, F^{\alpha\beta}\, \mathrm{d}t_c \mathrm{d}t_o = \int_0^\infty \int_0^\infty \sum_{i=1}^{I} \sum_{j=1}^{J} a_{ij} f_{ij}\, F^{\alpha\beta} \mathrm{d}t_c \mathrm{d}t_o$$

$$= \sum_{i=1}^{I} \sum_{j=1}^{J} a_{ij} \int_0^\infty \int_0^\infty f_{ij}\, F^{\alpha\beta} \mathrm{d}t_c \mathrm{d}t_o = a_{\alpha\beta}. \quad (8)$$

The integration carried out in Eq. 8 is valid for every pair of integer values that α and β may assume in the ranges 1 to I and 1 to J, respectively. All of the coefficients a_{ij} may thus be evaluated.

STATISTICAL VARIANCE OF THE VALUES OF THE CALCULATED COEFFICIENTS

The method described above for the evaluation of the parameters a_{ij} has potentially unlimited precision if the true probability–density distribution functions are introduced in the integration procedure required by Eq. 8. This idealized case would have been realized if the single-channel recording were extended indefinitely, and the number of closed–open pair events available for analysis were infinitely large. Under such circumstances the histograms yielded by the data coincide with the true probability density distribution functions. In practice such a situation cannot be attained, of course. Due to the stochastic nature of the occurrence of intervals of specified duration in the single-channel recordings, there is a nonvanishing likelihood that an error will be introduced in the analysis of the data when the number of events available for analysis is finite. If the values estimated for the parameters a_{ij} are to be meaningful it is necessary to have an estimate of the range of error that the calculated values of the coefficients may have. The above method for the calculation of the values of a_{ij} conveniently affords a facile procedure for the evaluation of the statistical variances to be expected for the calculated values of a_{ij} given the limitations in the amount of the available data.

Let us first calculate the value of the variance if we have only a single event of a pair of closed–open durations at our disposal for analysis. This pair has a probability $p(t_c, t_o)$ to be of durations t_c and t_o; thus for this pair of intervals the square deviation in the evaluation of a_{ij} is $[F^{ij}(t_c, t_o) - a_{ij}]^2$. To obtain the value of the variance, $\mathrm{var}_{ij}(1)$, in a_{ij} when a single event is available, one should integrate, with proper weighting, over all values of t_c and t_o:

$$\mathrm{var}_{ij}(1) = \int_0^\infty \int_0^\infty p(t_c, t_o) \cdot (F^{ij} - a_{ij})^2\, \mathrm{d}t_c \mathrm{d}t_o. \quad (9)$$

For conciseness, $F^{ij}(t_c, t_o)$ has been replaced by F^{ij} in Eq. 9.

If the number of events, N, which are available for analysis is large compared to the number of events in a closed–open sequence over which correlations between durations practically vanish (which is of the order of just a few events, see for example Labarca et al., 1985), we may justifiably assume that the great majority of events are not correlated. Furthermore, even neighboring events are not expected to correlate very strongly. Thus, in the case of the acetylcholine-activated channel, consecutive open durations separated by a single closed duration were found to correlate to an extent of <30%, and further-removed open durations correlated to a much lesser extent (Labarca et al., 1985). We may therefore use the following expression, which applies to noncorrelated events, as an approximated estimate for the statistical variance, $\mathrm{var}_{ij}(N)$, in the calculated values of the coefficients describing the histogram of events when N events are available for analysis[1]:

$$\mathrm{var}_{ij}(N) \simeq N \cdot \mathrm{var}_{ij}(1) = N \cdot \int_0^\infty \int_0^\infty p(t_c, t_o) \cdot (F^{ij} - a_{ij})^2\, \mathrm{d}t_c \mathrm{d}t_o = N \left\{ \int_0^\infty \int_0^\infty p(t_c, t_o) \cdot (F^{ij})^2\, \mathrm{d}t_c \mathrm{d}t_o - \left[\int_0^\infty \int_0^\infty p(t_c, t_o) \cdot F^{ij}\, \mathrm{d}t_c \mathrm{d}t_o \right]^2 \right\}. \quad (10)$$

Eq. 10 applies to the analysis of a non-normalized histogram of events as a function of t_c and t_o, where every event is assigned a numerical magnitude of unity. If one uses as input data a normalized histogram of events, in which the frequencies sum up to unity, then each event has a numerical magnitude of $1/N$, and F^{ij}, which appears in Eq. 10, has to be substituted by F^{ij}/N. The corresponding expression for the statistical variance assumes the following form:

$$\mathrm{var}_{ij}(N)(\text{normalized}) \simeq (1/N) \left\{ \int_0^\infty \int_0^\infty p(t_c, t_o) \cdot (F^{ij})^2\, \mathrm{d}t_c \mathrm{d}t_o - \left[\int_0^\infty \int_0^\infty p(t_c, t_o) \cdot F^{ij}\, \mathrm{d}t_c \mathrm{d}t_o \right]^2 \right\}. \quad (10a)$$

The function $p(t_c, t_o)$, substituted by the normalized histogram of events, is of course only approximate in view of the finite number of events that is experimentally available, but the data should usually be sufficient to yield acceptable estimates of the variances. Obviously, the variance will depend in each case on the particular function $F^{\alpha\beta}(t_c, t_o)$, as well as on $p(t_c, t_o)$.

EXAMPLE

For the sake of illustration, the functions $F^{\alpha\beta}(t_c, t_o)$ will be expressed explicitly by use of Eq. 7 for the special case in which there are two nonconducting states and two conducting states, i.e., $I = J = 2$. The functions will then be numerically evaluated for a set of lifetimes chosen from the published literature. A computer-simulated experiment will be performed in which events are assigned to the t_c–t_o plane in such a way that the distribution conforms to a probability–density distribution function with the above lifetimes as parameters. The coefficients a_{ij} in this density distribution function will then be extracted from these computer-generated data with the aid of the auxiliary functions $F^{\alpha\beta}(t_c, t_o)$ by use of Eq. 8. The deviations obtained in the values of a_{ij} will be shown to be within the limits predicted by the statistical variances estimated by use of Eq. 10a.

For the case that $I = J = 2$, Eq. 7 can be rewritten in detailed form as follows, using the abbreviated notation

$$\int_0^\infty \int_0^\infty f_{ij} f_{mn}\, dt_c dt_o = \langle f_{ij} | f_{mn} \rangle :$$

$$b_{11}^{\alpha\beta}\langle f_{11}|f_{11}\rangle + b_{12}^{\alpha\beta}\langle f_{12}|f_{11}\rangle + b_{21}^{\alpha\beta}\langle f_{21}|f_{11}\rangle + b_{22}^{\alpha\beta}\langle f_{22}|f_{11}\rangle = \delta_1^\alpha \cdot \delta_1^\beta$$

$$b_{11}^{\alpha\beta}\langle f_{11}|f_{12}\rangle + b_{12}^{\alpha\beta}\langle f_{12}|f_{12}\rangle + b_{21}^{\alpha\beta}\langle f_{21}|f_{12}\rangle + b_{22}^{\alpha\beta}\langle f_{22}|f_{12}\rangle = \delta_1^\alpha \cdot \delta_2^\beta$$

$$b_{11}^{\alpha\beta}\langle f_{11}|f_{21}\rangle + b_{12}^{\alpha\beta}\langle f_{12}|f_{21}\rangle + b_{21}^{\alpha\beta}\langle f_{21}|f_{21}\rangle + b_{22}^{\alpha\beta}\langle f_{22}|f_{21}\rangle = \delta_2^\alpha \cdot \delta_1^\beta$$

$$b_{11}^{\alpha\beta}\langle f_{11}|f_{22}\rangle + b_{12}^{\alpha\beta}\langle f_{12}|f_{22}\rangle + b_{21}^{\alpha\beta}\langle f_{21}|f_{22}\rangle + b_{22}^{\alpha\beta}\langle f_{22}|f_{22}\rangle = \delta_2^\alpha \cdot \delta_2^\beta. \quad (11)$$

Note that Eq. 11 represents four sets of four equations each, where each of α's and β's can assume a value of 1 or 2. Each set of four equations can be solved to yield the coefficients $b_{ij}^{\alpha\beta}$ that are necessary for the representation of $F^{\alpha\beta}$ by use of Eq. 6.

Solving the integrals represented by $\langle f_{ij}|f_{mn}\rangle$ analytically yields the following identities for $i = 1$ or 2 and $j = 1$ or 2:

$$\langle f_{ij}|f_{ij}\rangle = \frac{1}{4\tau_i\theta_j}$$

$$\langle f_{ij}|f_{in}\rangle = \frac{1}{4\tau_i\theta_1\theta_2\sigma_o}, \quad \text{for } j \neq n$$

$$\langle f_{ij}|f_{mj}\rangle = \frac{1}{4\tau_1\tau_2\theta_j\sigma_c}, \quad \text{for } i \neq m$$

$$\langle f_{ij}|f_{mn}\rangle = \frac{1}{4\tau_1\tau_2\theta_1\theta_2\sigma_c\sigma_o}, \quad \text{for } i \neq m \text{ and } j \neq n, \quad (12)$$

where,

$$\sigma_c = (1/2)(1/\tau_1 + 1/\tau_2)$$

$$\sigma_o = (1/2)(1/\theta_1 + 1/\theta_2). \quad (13)$$

Substitution in Eq. 11 and solution of the resulting linear simultaneous equations for $b_{11}^{\alpha\beta}$, $b_{12}^{\alpha\beta}$, $b_{21}^{\alpha\beta}$ and $b_{22}^{\alpha\beta}$ by standard techniques yields the following result:

$$\begin{pmatrix} b_{11}^{\alpha\beta} \\ b_{12}^{\alpha\beta} \\ b_{21}^{\alpha\beta} \\ b_{22}^{\alpha\beta} \end{pmatrix} = \Phi \begin{pmatrix} \tau_1\theta_1 & -\tau_1/\sigma_o & -\theta_1/\sigma_c & 1/(\sigma_c\sigma_o) \\ -\tau_1/\sigma_o & \tau_1\theta_2 & 1/(\sigma_c\sigma_o) & -\theta_2/\sigma_c \\ -\theta_1/\sigma_c & 1/(\sigma_c\sigma_o) & \tau_2\theta_1 & -\tau_2/\sigma_o \\ 1/(\sigma_c\sigma_o) & -\theta_2/\sigma_c & -\tau_2/\sigma_o & \tau_2\theta_2 \end{pmatrix} \begin{pmatrix} \delta_1^\alpha\delta_1^\beta \\ \delta_1^\alpha\delta_2^\beta \\ \delta_2^\alpha\delta_1^\beta \\ \delta_2^\alpha\delta_2^\beta \end{pmatrix}, \quad (14)$$

where,

$$\phi = 64\{\sigma_c\sigma_o/[(1/\tau_1 - 1/\tau_2)(1/\theta_1 - 1/\theta_2)]\}^2. \quad (15)$$

It may be noted that ϕ times the square matrix in Eq. 14 is the inverse of the matrix of the coefficients for $b_{ij}^{\alpha\beta}$ in Eq. 11. Obviously, the parameters b_{ij}^{11} correspond to the first column in the matrix in Eq. 14, b_{ij}^{12} to the second column, and so on. One thus obtains the following expressions for $F^{\alpha\beta}$:

$$F^{11} = \Phi[f_{11}\tau_1\theta_1 - f_{12}\tau_1/\sigma_o - f_{21}\theta_1/\sigma_c + f_{22}/(\sigma_c\sigma_o)]$$

$$F^{12} = \Phi[-f_{11}\tau_1/\sigma_o + f_{12}\tau_1\theta_2 + f_{21}/(\sigma_c\sigma_o) - f_{22}\theta_2/\sigma_c]$$

$$F^{21} = \Phi[-f_{11}\theta_1/\sigma_c + f_{12}/(\sigma_c\sigma_o) + f_{21}\tau_2\theta_1 - f_{22}\tau_2/\sigma_o]$$

$$F^{22} = \Phi[f_{11}/(\sigma_c\sigma_o) - f_{12}\theta_2/\sigma_c - f_{21}\tau_2/\sigma_o + f_{22}\tau_2\theta_2]. \quad (16)$$

By substitution of the various $F^{\alpha\beta}$ functions from Eq. 16 into Eq. 7 and subsequent integration it may be readily verified that the equalities of Eq. 7 are fulfilled for all numerical values assigned to the lifetimes (except for undefined results if $\tau_1 = \tau_2$ or $\theta_1 = \theta_2$, where $\Phi = \infty$, see Eq. 15. It is however futile to define two identical lifetimes when a single lifetime describes the data). The equalities presented in Eq. 16 may thus be readily used in cases in which the probability–density distribution functions of each of the closed and open intervals are comprised of two exponential decay functions. In more complex cases, the larger set of $F^{\alpha\beta}$ functions that is required may be obtained by following an exactly analogous procedure to the one described above. For a given set of lifetimes expressed numerically, the numerical matrix of coefficients of $b_{ij}^{\alpha\beta}$ that appears in Eq. 11 may be readily inverted by computer by use of available software.

The above procedure will be further pursued with a numerical example using values of lifetimes picked from the published literature. Jackson et al. (1983) have resolved into biexponential functions the closed-time durations and the open-time durations of the acetylcholine receptor of cultured rat skeletal muscle. The cumulative distributions of the conducting durations had lifetimes of 12.52 ms (120 events) and 0.36 ms (691 events), and that of the nonconducting durations had lifetimes of 20.16 ms (40 events) and 0.5 ms (100 events). (The later numbers were not stated explicitly in Jackson et al., 1983, but were deduced from Fig. 1 of the paper, and are necessarily approximate). The various f_{ij} functions may thus be expressed as follows,

$$f_{11} = (1/20.16)(1/12.52)\exp(-t_c/20.16) \cdot \exp(-t_o/12.52)$$

$$f_{12} = (1/20.16)(1/0.36) \cdot \exp(-t_c/20.16)\exp(-t_o/0.36)$$

$$f_{21} = (1/0.5)(1/12.52)\exp(-t_c/0.5)\exp(-t_o/12.52)$$

$$f_{22} = (1/0.5)(1/0.36)\exp(-t_c/0.5)\exp(-t_o/0.36). \quad (17)$$

These functions are plotted in Fig. 1.

The $F^{\alpha\beta}$ functions can be readily expressed by use of Eq. 16 and are shown in graphical form in Fig. 2. These functions can then be used to evaluate the coefficients of f_{ij} in any arbitrary given function,

$$p(t_c, t_o) = \sum_{i=1}^{2}\sum_{j=1}^{2} a_{ij}f_{ij}. \quad (18)$$

The a_{ij} values reflect, among other things, the correlations between the durations of consecutive intervals in the single-channel recording. In the case of the acetylcholine channel, such correlations were definitely demonstrated but were not formulated in complete detail. Because of the unavailability of reported quantitative data, the following values for a_{ij} were arbitrarily chosen for the computer-simulated experiment to be presented:

$$a_{11} = 0.04228$$

$$a_{12} = 0.24344$$

$$a_{21} = 0.10569$$

$$a_{22} = 0.60860. \quad (19)$$

These values can be shown to correspond to a case in which

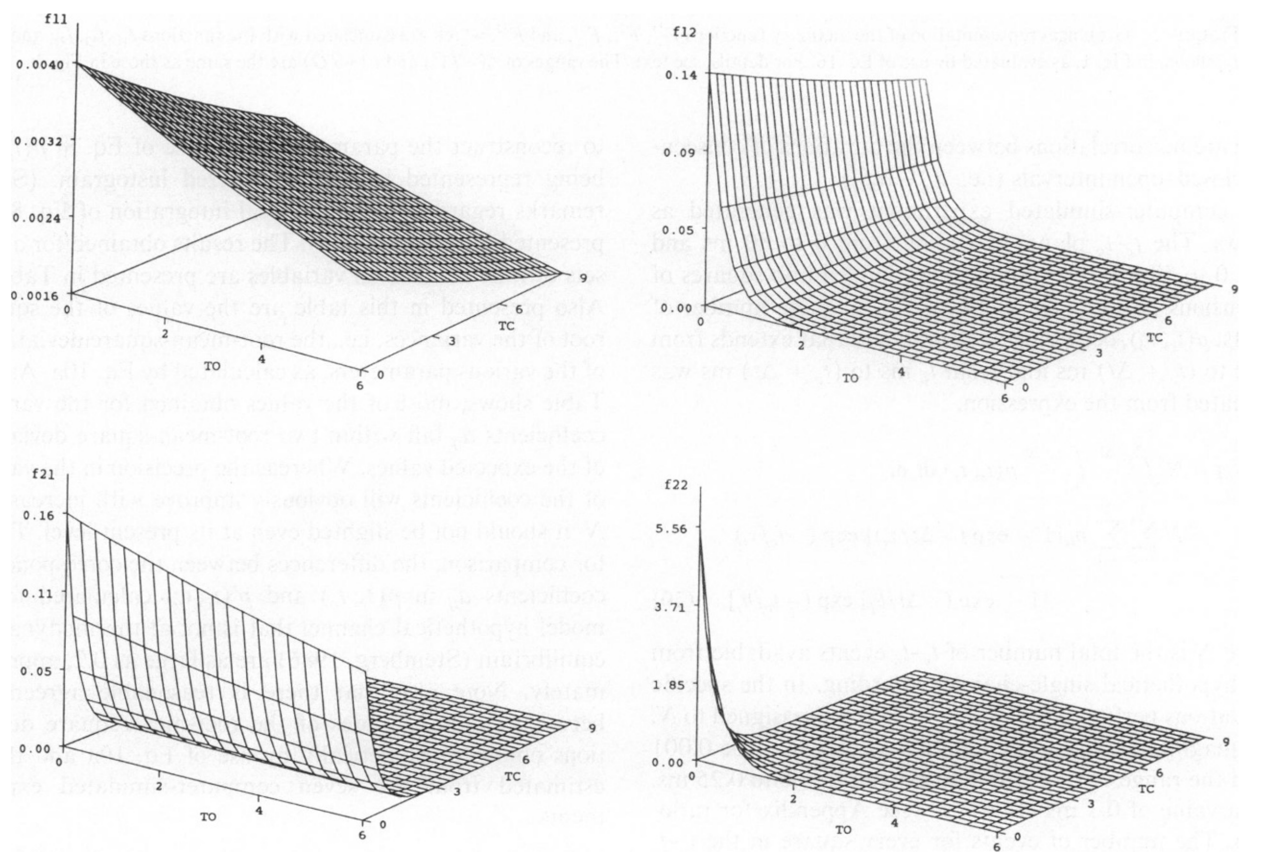

FIGURE 1 Graphical representation of the functions f_{11}, f_{12}, f_{21}, and f_{22} (see Eq. 17) used for the computer-simulated example. The ranges of $t_c(=TC)$ and $t_o(=TO)$ in the figure were limited to 0–9 ms and 0–6 ms, respectively, to accentuate the steep changes that occur in these time ranges.

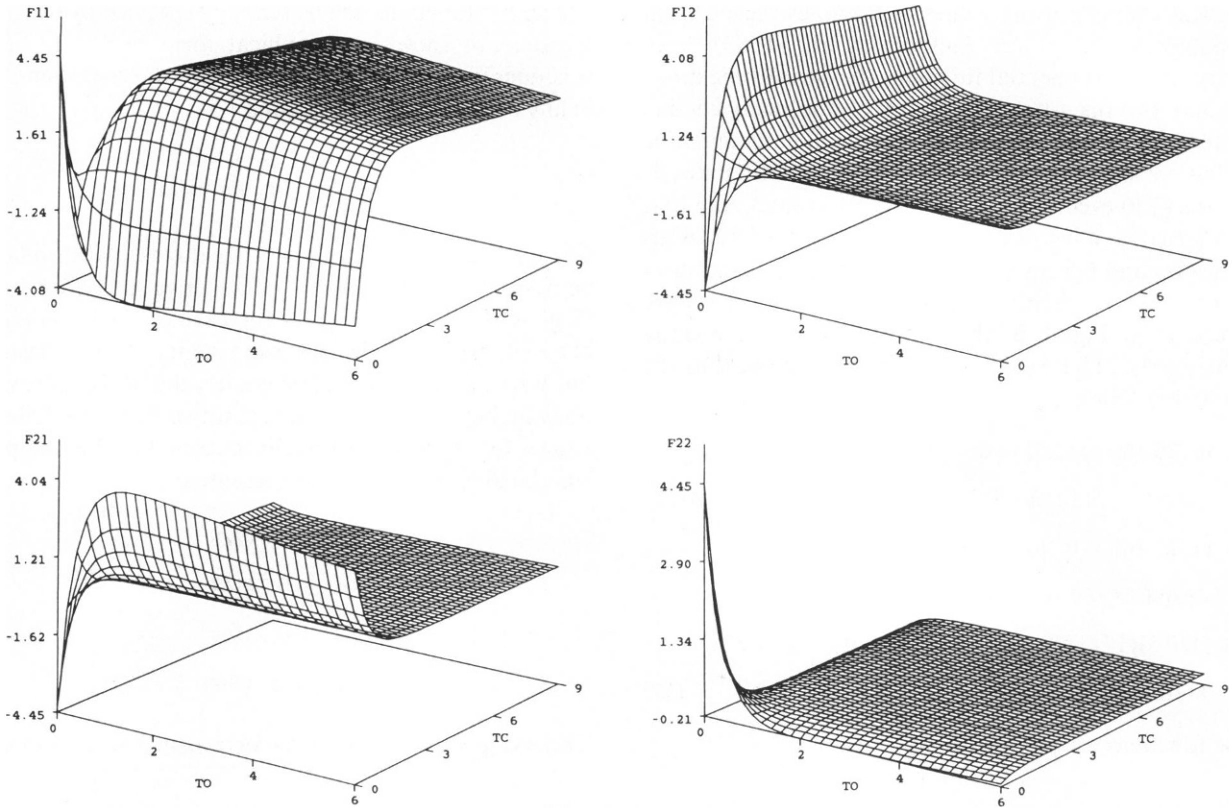

FIGURE 2 Graphical representation of the auxiliary functions F^{11}, F^{12}, F^{21}, and F^{22}, which are associated with the functions f_{11}, f_{12}, f_{21}, and f_{22} shown in Fig. 1, as evaluated by use of Eq. 16. For details, see text. The ranges of $t_c(=TC)$ and $t_o(=TO)$ are the same as those in Fig. 1.

there are no correlations between the durations of consecutive closed–open intervals (i.e., $a_{ij} = \alpha_i\beta_j$).

A computer-simulated experiment was generated as follows. The t_c–t_o plane, extending from 0 to 80 ms and from 0 to 50 ms, respectively, was divided into squares of dimensions Δt ms · Δt ms each. The average number of events, $\mu(t_c, t_o)$, occurring in each square that extends from t_c ms to $(t_c + \Delta t)$ ms and from t_o ms to $(t_o + \Delta t)$ ms was evaluated from the expression,

$$\mu(t_c, t_o) = N \int_{t_c}^{t_c+\Delta t} \int_{t_o}^{t_o+\Delta t} p(t_c, t_o)\, \mathrm{d}t_c \mathrm{d}t_o$$

$$= N \sum_{i=1}^{2} \sum_{j=1}^{2} a_{ij}[1 - \exp(-\Delta t/\tau_i)] \exp(-t_c/\tau_i) \cdot [1 - \exp(-\Delta t/\theta_j] \exp(-t_o/\theta_j], \quad (20)$$

where N is the total number of t_c–t_o events available from the hypothetical single-channel recording. In the specific simulations performed, a value of 1,000 was assigned to N. The magnitude of the interval Δt was chosen to be 0.001 ms in the range of $t_c = 0$ to 0.40 ms and $t_o = 0$ to 0.25 ms, and a value of 0.1 ms elsewhere (see Appendix for rationale). The number of events for every square in the t_c–t_o plane was randomly assigned, subject to a Poisson distribution function with an average value $\mu(t_c, t_o)$ as obtained by use of Eq. 20. The histogram thus obtained was normalized by division by N and used as simulated experimental data to reconstruct the parameters a_{ij} by use of Eq. 8, $p(t_c, t_o)$ being represented by the normalized histogram. (Some remarks regarding the numerical integration of Eq. 8 are presented in the Appendix). The results obtained for a few sets of random Poisson variables are presented in Table I. Also presented in this table are the values of the square root of the variances, i.e., the root-mean-square deviations, of the various parameters, as calculated by Eq. 10a. As the Table shows, most of the values obtained for the various coefficients a_{ij} fall within two root-mean-square deviation of the expected values. Whereas the precision in the values of the coefficients will obviously improve with increase in N, it should not be slighted even at its present level. Thus, for comparison, the differences between the corresponding coefficients a_{ij} in $p(t_c, t_o)$ and $p'(t_c, t_o)$ calculated for a model hypothetical channel that is not at thermodynamic equilibrium (Steinberg, 1986) are as large as 0.2, approximately. Note also that there is reasonable agreement between the magnitudes of the root-mean-square deviations obtained theoretically by use of Eq. 10a and those estimated from the seven computer-simulated experiments.

DISCUSSION

A simple procedure was presented above for the analysis of data obtained from single-channel recordings directed

TABLE I

EVALUATION OF THE COEFFICIENTS a_{ij} FROM COMPUTER-SIMULATED EXPERIMENTS

	Input values	Values derived from computer experiments*							"Experimental"[‡] RMS	Theoretical[‡§] RMS
		I	II	III	IV	V	VI	VII		
a_{11}	0.042	0.001	0.129	0.113	0.024	0.066	0.054	0.060	0.0417	0.0453
a_{12}	0.243	0.224	0.200	0.215	0.252	0.197	0.220	0.324	0.0415	0.0416
a_{21}	0.106	0.105	0.095	0.057	0.138	0.097	0.138	0.104	0.0252	0.0384
a_{22}	0.609	0.620	0.627	0.627	0.545	0.606	0.564	0.165	0.0201	0.0347

*The Roman numerals are used to identify the various computer-simulated experiments.

[‡]RMS is the root-mean-square deviation, i.e., the square root of the variance, of the corresponding coefficients.

[§]Calculated by use of Eq. 10a.

toward the evaluation of the probability–density distribution functions $p(t_c, t_o)$ and $p^r(t_c, t_o)$ for the occurrence of t_c–t_o and t_o–t_c consecutive durations, respectively. The procedure requires a preprocessing of the closed durations and the open durations separately in terms of the relevant time constants, before the evaluation of the parameters a_{ij} that appear in the above distribution functions. This is of obvious advantage over a global analysis performed in a single step, since in the procedure proposed above one minimizes compensating effects among the time constants for closed and open durations and the a_{ij} parameters during the analysis. The procedure described above also permits the evaluation of the statistical error that may occur in the calculated numerical values of the parameters a_{ij} in the probability–density distribution functions. This is of obvious importance for defining the limits of the quantitative significance of the values obtained.

No detailed model for the kinetic behavior of the channel studied was assumed in the above analysis. The results of the analysis may, however, be very useful for gaining information about the kinetic scheme that describes the behavior of the channel, since this scheme determines the values of the various coefficients a_{ij} that appear in the expressions for $p(t_c, t_o)$. These parameters thus put restrictions on proposed models additionally to those provided by the values of the lifetimes τ_i and θ_j and the preexponents α_i and β_j. The relationships between the various parameters a_{ij} and the rate constants that appear in the kinetic scheme of the opening and closing of the channel are as follows (Steinberg, 1986):

$$a_{ij} = \left(\sum_{i=1}^{I} \sum_{j=1}^{J} \Phi_{ij}\right)^{-1} \cdot \Phi_{ij}, \tag{21}$$

where Φ_{ij} is the rate of interconversion of the component of the aggregate of closed states that decays with the lifetime τ_i into the component of the aggregate of open states that decays with the lifetime θ_j. Φ_{ij} is given by,

$$\Phi_{ij} = [\underset{\sim}{u}]\underset{\sim}{R}^{-1}[\delta_{jj}]\underset{\sim}{R}\underset{\sim}{K}^T\underset{\sim}{Q}^{-1}[\delta_{ii}]\underset{\sim}{Q}\underset{\sim}{V}\{\underset{\sim}{u}\}, \tag{22}$$

where $[\underset{\sim}{u}]$ and $\{\underset{\sim}{u}\}$ are raw and column matrices, respectively, all elements of which are unity; $\underset{\sim}{V}$ is a diagonal matrix, the elements v_{ii} of which represent the equilibrium concentrations of the closed states i in an ensemble of channels; $\underset{\sim}{Q}$ and $\underset{\sim}{Q}^{-1}$ are the matrices that diagonalize the transpose of the matrix $\underset{\sim}{G}$ of the rate constants g_{ix} that characterize the kinetics of the disappearance of the various states in an aggregate of closed states; $\underset{\sim}{R}$ and $\underset{\sim}{R}^{-1}$ are the matrices that diagonalize the transpose of the matrix $\underset{\sim}{H}$ of the rate constants h_{jy} that characterize the kinetics of the disappearance of the various states in an aggregate of open states; $[\delta_{ii}]$ and $[\delta_{jj}]$ are square matrices of dimensions I and J, respectively, in which all elements are zero except elements ii and jj, respectively, which are unity; and $\underset{\sim}{K}^T$ is the transpose of the matrix $\underset{\sim}{K}$ of the rate constants k_{ij} for the conversion of closed state i into open state j of the channel. Combining Eqs. 21 and 22, one may express a_{ij} as follows,

$$a_{ij} = ([\underset{\sim}{u}]\underset{\sim}{K}^T\underset{\sim}{V}\{\underset{\sim}{u}\})^{-1}[\underset{\sim}{u}]\underset{\sim}{R}^{-1}[\delta_{jj}]\underset{\sim}{R}\underset{\sim}{K}^T\underset{\sim}{Q}^{-1}[\delta_{ii}]\underset{\sim}{Q}\underset{\sim}{V}\{\underset{\sim}{u}\}. \tag{23}$$

The corresponding parameters a^r_{ji} in the probability density distribution function $p^r(t_c, t_o)$ for the occurrence of an open duration t_o followed by a closed duration t_c are given by,

$$a^r_{ji} = ([\underset{\sim}{u}]\underset{\sim}{L}^T\underset{\sim}{W}\{\underset{\sim}{u}\})^{-1}[\underset{\sim}{u}]\underset{\sim}{Q}^{-1}[\delta_{ii}]\underset{\sim}{Q}\underset{\sim}{L}^T\underset{\sim}{R}^{-1}[\delta_{jj}]\underset{\sim}{R}\underset{\sim}{W}\{\underset{\sim}{u}\}, \tag{24}$$

where $\underset{\sim}{W}$ is a diagonal matrix, the elements w_{jj} of which represent the equilibrium concentrations of the open states j in an ensemble of channels; and $\underset{\sim}{L}^T$ is the transpose of the matrix $\underset{\sim}{L}$ of the rate constants ℓ_{ji} for the conversion of open state j into closed state i of the channel. Thus, a_{ij} and a^r_{ji} can be evaluated for any model proposed for the channel conductance changes, and the calculated values of these parameters can be compared to the values obtained from the single-channel recording as prescribed above. Note that if the channel behaves like a system at thermodynamic equilibrium, i.e., if it fulfills the condition of detailed balance, then $a_{ij} = a^r_{ji}$ (Steinberg, 1986). The evaluation of a_{ij} and a^r_{ji} from the single channel recordings may thus be of much importance in shedding light on the thermodynamic state of the ion channel, and in indicating whether its gating is coupled to dissipative processes.

The significance of Eqs. 23 and 24 is that they relate the parameters a_{ij} and a^r_{ji} to the individual rate constants k_{ij} and ℓ_{ji}, whereas the matrices $\underset{\sim}{G}$ and $\underset{\sim}{H}$, which determine τ_i

and θ_j, contain only sums of these rate constants ($\Sigma_{j=1}^{J} g_{ij}$ and $\Sigma_{i=1}^{I} h_{ji}$, respectively). The evaluation of these parameters from the measured data thus puts severe experimental restrictions on any proposed kinetic model. Moreover, as Fredkin et al. (1985) have shown, knowledge of a_{ij} and a'_{ji} is sufficient to describe the statistical behavior of any length of a single-channel trace; no new information is thus obtained in principle from analysis of sequences that contain more than pairs of intervals (although such extended analyses may sometimes be of value for practical reasons).

As an example let us examine the following scheme for the ion channel:

$$c_1 \rightleftarrows c_2 \rightleftarrows d_2 \rightleftarrows d_1, \qquad (25)$$

where c_1 and c_2 denote nonconducting channel states, and d_1 and d_2 denote conducting states. This scheme is equivalent to scheme I examined by Jackson et al. (1983) as a candidate model for the channel activated by acetylcholine. The matrix $\underset{\sim}{K}$ thus assumes the form

$$\underset{\sim}{K} = \underset{\sim}{K}^T = \begin{pmatrix} 0 & 0 \\ 0 & k_{22} \end{pmatrix} = \begin{pmatrix} 0 \\ 1 \end{pmatrix} \cdot (0 \; k_{22}). \qquad (26)$$

Substitution of Eq. 26 into Eq. 23 yields the following expression for the parameters a_{ij}:

$$a_{ij} = ([\underset{\sim}{u}]\underset{\sim}{K}^T\underset{\sim}{V}\{\underset{\sim}{u}\})^{-1}[\underset{\sim}{u}]\underset{\sim}{R}^{-1}[\delta_{jj}]R\begin{pmatrix} 0 \\ 1 \end{pmatrix} \cdot (0 \; k_{22})\underset{\sim}{Q}^{-1}[\delta_{ii}]\underset{\sim}{Q}\underset{\sim}{V}\{\underset{\sim}{u}\}. \qquad (27)$$

The parameters a_{ij} may thus be factorized into a product of two scalars. Note that similar factorization may be carried out for any matrix $\underset{\sim}{K}^T$ for which $k_{11}/k_{21} = k_{12}/k_{22}$, where

$$\underset{\sim}{K}^T = \begin{pmatrix} k_{11} \\ k_{12} \end{pmatrix} \cdot (1 \; k_{21}/k_{11}) = \begin{pmatrix} k_{21} \\ k_{22} \end{pmatrix} \cdot (k_{11}/k_{21} \; 1). \qquad (28)$$

This condition is fulfilled, for example, by scheme III of Jackson et al. (1983). It thus follows that for the scheme shown in Eq. 25, as well as for all other cases in which $\underset{\sim}{K}^T$ can be written by the form shown in Eq. 28, the following relationship should hold between the various a_{ij} coefficients,

$$a_{11} \cdot a_{22} = a_{12} \cdot a_{21}. \qquad (29)$$

Substitution of this relationship into the expression for $p(t_c, t_o)$ reveals that it is a mathematical formulation of the fact, observed by Jackson et al. (1983), that the scheme presented in Eq. (25) dictates a random relationship between the durations of neighboring intervals in the single-channel recording. These authors used a simple approximate classification scheme of fast and slow transitions for the analysis of their data. As they point out, their scheme of classification was successful because of the great difference in relaxation times of the processes that operate in the case of the acetylcholine receptor channel. In more equivocal situations, they remark, testing of correlations would require a more elaborate theoretical framework. The method of analysis presented above proposes one such framework.

It is pertinent to note that although explicit reference was made above to the statistics of closed–open pair durations, the method of analysis is not limited to this specific kind of event. With no modification, except for obvious changes in notation, the method may also be used for the analysis of open–open durations separated by a closed duration or closed–closed durations separated by an open duration, since the probability–density functions for such pair events are also linear combinations of products of exponential functions. The correlations between open durations separated by intervening single closed durations were, for example, of prime interest in the study of the acetylcholine receptor channel by Jackson et al. (1983).

APPENDIX

A Note Regarding the Numerical Representation of the Functions f_{ij} and the Numerical Integration of Eq. 8

In the computer simulations the t_c–t_o plane was divided into squares of dimensions Δt ms · Δt ms. The value $f'_{ij}(t_c, t_o)$ that was numerically assigned to a function f_{ij} for a given square was,

$$\begin{aligned} f'_{ij}(t_c, t_o) &= (\Delta t)^{-2} \int_{t_c}^{t_c+\Delta t} \int_{t_o}^{t_o+\Delta t} f_{ij}\, dt_c dt_o \\ &= (\Delta t)^{-2}[1 - \exp(-\Delta t/\tau_i)][1 - \exp(-\Delta t/\theta_j)] \\ &\quad \cdot \exp(-t_c/\tau_i)\exp(-t_o/\theta_j). \end{aligned} \qquad (30)$$

This is the average value of the function at the square bounded by t_c to $t_c + \Delta t$ and t_o to $t_o + \Delta t$. Use of $f'_{ij}(t_c, t_o)$ ensures that the integral of f_{ij} over the t_c–t_o plane is unity within the roundoff error of the computations. However, this does not ensure that the integration of Eq. 8 is error free, since Eq. 8 requires the integration of products of the exponential functions as represented by Eq. 30.

It can be readily shown that when an integral of the type

$$\int_0^\infty \int_0^\infty f_{ij} f_{mn}\, dt_c\, dt_o$$

is substituted by a summation of the discrete function $f'_{ij}(t_c, t_o)f'_{mn}(t_c, t_o)$ as defined by Eq. 30, a relative error, *RE*, of magnitude

$$RE \simeq (1/4)[(\Delta t)^2/\tau_i\tau_m + (\Delta t)^2/\theta_j\theta_m] \qquad (31)$$

is introduced. Eq. 31 may be used as a guide for the choice of Δt to keep the integration errors within tolerable limits. Integration of Eq. 8 involves the integration of a variety of exponentials, and for this reason the values of Δt were chosen to be of different magnitude in different regions of the t_c–t_o plane.

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REFERENCES

Colquhoun, D., and A. G. Hawkes. 1977. Relaxation and fluctuation in membrane currents that flow through drug-operated channels. *Proc. R. Soc. Lond. B Biol. Sci.* 199:231–262.

Colquhoun, D., and A. G. Hawkes. 1981. On the stochastic properties of single ion channels. *Proc. R. Soc. Lond. B Biol. Sci.* 211:205–235.

Colquhoun, D., and F. J. Sigworth. 1983. Fitting and statistical analysis of single-channel recordings. *In* Single-Channel Recording. Sakmann B. and E. Neher, editors. Plenum Press, New York. 191–263.

Fredkin, D. R., M. Montal, and J. A. Rice. 1985. Identification of aggregated markovian models: application to the nicotinic acetylcholine receptor. *In* Proceedings of the Berkeley Conference in Honor of Jerzy Newman and Jack Kiefer. Wadsworth Publishing Co., Belmont, CA. 269–289.

Grinvald, A., and I. Z. Steinberg. 1974. On the analysis of fluorescence decay kinetics by the method of least-squares. *Anal. Biochem.* 59:583–598.

Hodgkin, A. L., and A. F. Huxley. 1952. A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol. (Lond.).* 117:500–544.

Jackson, M. B., B. S. Wong, C. E. Morris, H. Lecar, and C. N. Christian. 1983. Successive openings of the same acetylcholine receptor channel are correlated in open time. *Biophys. J.* 42:109–114.

Labarca, P., J. A. Rice, D. R. Fredkin, and M. Montal. 1985. Kinetic analysis of channel gating. Application to the cholinergic receptor channel and the chloride channel from *Torpedo californica. Biophys. J.* 47:469–478.

Sigworth, F. J. 1983. An example of analysis. *In* Single-Channel Recording. Sakmann B. and E. Neher, editors. Plenum Press, New York. 301–321.

Steinberg, I. Z. 1987. Relationship between statistical properties of single ionic channel recordings and the thermodynamic state of the channels. *J. Theor. Biol.* 124:71-87.