THEORY OF THE KINETIC ANALYSIS OF PATCH-CLAMP DATA

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ABSTRACT This paper describes a theory of the kinetic analysis of patch-clamp data. We assume that channel gating is a Markov process that can be described by a model consisting of n kinetic states and $n(n-1)$ rate constants at each voltage, and that patch-clamp data describe the occupancy of x different conductance levels over time. In general, all the kinetic information in a set of patch-clamp data is found in either two-dimensional dwell time histograms describing the frequency of observation of sequential dwell times of durations τ_1 and τ_2 (Fredkin, D. R., M. Montal, and J. A. Rice, 1985, Proceedings of the Berkeley Conference in Honor of Jerzy Neyman and Jack Kiefer, vol. 1, 269–289) or in three-point joint probability functions describing the probability that a channel is in a given conductance at time t, and at time $t + \tau_1$, and at time $t + \tau_1 + \tau_2$. For the special case of channels with a single open state plus multiple closed states, one-dimensional analyses provide all of the kinetic information. Stationary patch-clamp data have information that can be used to determine H rate constants, where $H = n(n-1) - G$ and G is the number of intraconductance rate constants. Thus, to calculate H rate constants, G rate constants must be fixed. In general there are multiple sets of G rate constants that can be fixed to allow the calculation of H rate constants although not every set of G rate constants will work. Arbitrary assignment of the G intraconductance rate constants equal to zero always provides a solution and the calculation of H rate constants. Nonstationary patch-clamp data have information for the determination of H rate constants at a reference voltage plus $n(n-1)$ rate constants at all test voltages. Thus, nonstationary data have extra information about the voltage dependencies of rate constants that can be used to rule out kinetic models that cannot be disqualified on the basis of stationary data.

INTRODUCTION

Recent advances in single channel recording techniques (Hamill et al., 1981) have made it possible to record the gating activity of single ion channels. A major goal of such measurements is the establishment of qualitative kinetic schemes that are consistent with the observed patterns of channel openings and closings and the quantitative measurement of the rate constants of appropriate kinetic models. To this end, Colquhoun and Hawkes (1977, 1981, 1982), Colquhoun and Sigworth (1983), Horn and Lange (1983), and others have developed methods for the kinetic analysis of patch-clamp data. These methods are based on the statistical analysis of dwell time measurements and offer practical methods for obtaining quantitative descriptions of channel gating. They do not, however, consider the number of independent rate constants that can be obtained or what sorts of qualitative kinetic schemes can and cannot be distinguished on the basis of patch-clamp data. Such questions were addressed in the work of Jackson et al. (1983), Kerry et al. (1986), Yeramian et al. (1986), and Blatz and Magleby (1986) who used covariance measurements to identify qualitatively the number of pathways between open and closed states. Similarly, Fredkin et al. (1985) and Colquhoun and Hawkes (1987) have shown how the correlation of dwell times can be used to distinguish between some types of valid and invalid kinetic schemes.

Recently, Fredkin et al. (1985) demonstrated that the maximum number of rate constants that can be determined from patch-clamp data showing two conductance levels was $2N_cN_o$, where N_c is the number of closed states and N_o is the number of open states. These results have been extended to include more than two conductance levels (Fredkin and Rice, 1986). Here, we present a more detailed derivation of similar results in terms of the number of rate constants that can be determined and we further consider the analysis of nonstationary data. In addition, we have determined the conditions under which this maximum number can be obtained.

We also describe an alternate approach to the analysis of patch-clamp data, namely the consideration of point joint probabilities instead of dwell times, and the development of mathematical constructs that have the kinetic information of patch-clamp data and are readily related to the eigen-

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vectors of rate constant matrices. This approach offers several important advantages. For example, in this paper, we demonstrate the use of this system to investigate the ability of patch-clamp data to distinguish between different kinetic models and have found that such data do not generally have information to identify pathways between open and closed states or the thermodynamics (i.e., detailed balance) of channel gating. Other advantages, including a method for the analysis of data containing more than one channel, and a simple numerical method for identifying kinetic schemes consistent with a set of patch-clamp data will be described in later papers (Bauer, R. J., and J. L. Kenyon, manuscripts in preparation).

PART I: GENERAL PROPERTIES OF PATCH-CLAMP DATA AND MARKOV STOCHASTIC PROCESSES

General Properties of Patch-Clamp Data

Patch-clamp data are presumed to record the flow of current through individual membrane channels over time, where the current flowing through any single channel is characteristically observed to fluctuate among x distinct levels. Transitions between these conductance levels are instantaneous (within the resolution of the measurement) and the data can be described as a series of sojourns in discrete conductance states. In addition to the distinguishable conductance levels, channel gating frequently shows evidence of multiple kinetic states of the same conductance level. Transitions among these states are not observed as changes in the channel current, rather they are identified by their impact on the kinetics of channel gating. In particular, the density functions (typically dwell time histograms) for each conductance level show an exponential relaxation for each kinetic state that contributes to that conductance (cf. Colquhoun and Sigworth, 1983). Thus, by inspection of patch-clamp data and appropriate density functions, one can determine the number of conductance levels (x) and the number of kinetic states (n) that characterize channel gating. The goal of kinetic analysis of patch-clamp data is to find as many of the rate constants as possible describing the transitions between the kinetic states that are consistent with the observed patterns of channel gating and the dependencies of these rate constants on various regulating factors. Because transitions within a conductance are not observed directly, when such transitions occur, there is ambiguity in patch-clamp data which precludes the determination of all $n(n-1)$ rate constants.

There are two kinds of patch-clamp data. Stationary patch-clamp data are collected over periods when the probabilities of occupancy of the individual kinetic states are constant. In contrast, to obtain information concerning the dependencies of rate constants on a regulatory factor, it is common to impose a step change of that factor and record channel activity over a period when the probabilities of occupancy of the individual kinetic states are changing, i.e., relaxing to new steady-state values determined by the new rate constants. Such data are nonstationary. By relating data obtained just before the jump with those obtained after the jump, one obtains information concerning the regulation of the rate constants. Below, we first consider the kinetic analysis of stationary patch-clamp data and then extend the analysis to nonstationary data. Because membrane potential is the most commonly studied regulatory factor, we have couched our discussion in terms of the voltage dependence of the rate constants. We point out here that our results apply to any regulatory factor that can be changed instantaneously while patch-clamp data are recorded.

We have kept our analysis general by limiting the number of assumptions and, in particular, we consider that channel gating shows n kinetic states and x conductance levels. We assume that channel gating is a Markov process that can be described by a model consisting of n kinetic states and $n(n-1)$ rate constants describing the instantaneous rates of transition between pairs of those states, and that the rate constants can be instantaneous functions of an experimentally manipulable parameter, such as voltage. Further, we assume that the number of kinetic states associated with each conductance level has been determined as described above.

Review of Markov Stochastic Processes

We review here the elements of the theory of Markov processes and matrix algebra and their application to the kinetic analysis of patch-clamp data. More complete discussions of the mathematics can be found in Karlin and Taylor (1975) and the application to patch-clamp data in Colquhoun and Hawkes (1977), Colquhoun and Sigworth (1983), and Fredkin et al. (1985).

The instantaneous rate of transition from some kinetic state i to a state j can be written as $q(i,j)$ and the rate constants of an n state kinetic scheme form an n by n matrix $\mathbf{Q}$ with the diagonals of the matrix, $q(i,i)$, given by

$$q(i,i) = -\sum_{\substack{j=1 \\ j\neq i}}^{n} q(i,j). \tag{1}$$

Thus, there are $n(n-1)$ independent elements in the n by n array. For a matrix $\mathbf{Q}$, a column eigenvector matrix $\mathbf{C}$, a diagonal eigenvalue matrix $\mathbf{\Lambda}$, and row eigenvector matrix $\mathbf{R}$ can be found such that

$$\mathbf{Q} = \mathbf{C\Lambda R}, \tag{2}$$

where

$$\mathbf{R} = \mathbf{C}^{-1}.$$

Thus

$$\mathbf{RC} = \mathbf{CR} = \mathbf{I}_d,$$

where $\mathbf{I}_d$ is the identity matrix. The elements of these matrices are designated $c(i, m)$ for matrix **C**, $r(m, j)$ for matrix **R**, and $\lambda(m)$, for the diagonal elements of matrix $\mathbf{\Lambda}$. For **R** to exist, it is sufficient that there are n distinct eigenvalues $\lambda(m)$. We stipulate that **Q** has n distinct eigenvalues. We also define $\mathbf{c}(m)$ as the mth column eigenvector to matrix **C** containing elements $c(i, m)$, associated with eigenvalue $\lambda(m)$, while $\mathbf{r}'(m)$ is the mth row eigenvector to matrix **R** containing elements $r(m, j)$, associated with eigenvalue $\lambda(m)$ (cf. Colquhoun and Hawkes, 1987).

In element form we can write

$$\sum_{m=1}^{n} c(i, m)r(m, j) = \delta(i, j) \tag{3}$$

or

$$\sum_{k=1}^{n} r(p, k)c(k, m) = \delta(p, m), \tag{4}$$

where $\delta(i, j) = 0$ for $i \neq j$, and $\delta(i, j) = 1$ for $i = j$. Because of Eq. 1, **Q** is singular, and one of the eigenvalues is zero. We designate

$$\lambda(n) = 0. \tag{5}$$

Since **Q** has distinct eigenvalues, there is only one zero eigenvalue and the rank of **Q** is $n - 1$. Only the relative values of the elements within each column vector are important in specifying **Q**. Thus for each column vector, one of the c elements can be given an arbitrary designation. For columns $m = 1$ to $n - 1$, a convenient designation is $c(i, m) = 1$ for some i. For the column n, we note that since $\mathbf{QC} = \mathbf{C\Lambda}$ by rearrangement of Eq. 2, then

$$\mathbf{Qc}(n) = \lambda(n)\mathbf{c}(n) = 0$$

or

$$\sum_{j=1}^{n} q(i, j)c(j, n) = 0. \tag{6}$$

By comparing Eq. 6 with Eq. 1, we see that $c(j, n) =$ constant is a solution and in particular we specify

$$c(j, n) = 1 \tag{7}$$

for all $j = 1$ to n.

These equations limit the number of independent eigenvector elements to specify **Q** to $(n - 1)$ $(n - 1)$. Together with $n - 1$ nonzero eigenvalues, they define the Markov system of $n(n - 1)$ independent rate constants via Eq. 2.

The conditional probability that a channel is in state j given that it was in state i at time τ earlier, $p(i, j, \tau)$, is related to the eigenvector and eigenvalue matrices described above by (Colquhoun and Hawkes, 1977; Karlin and Taylor, 1975)

$$\mathbf{P} = \exp(\mathbf{Q}\tau) = \mathbf{C}\exp(\mathbf{\Lambda}\tau)\mathbf{R}. \tag{8}$$

For convenience we define

$$\mathbf{G}(\tau) = \exp(\mathbf{\Lambda}\tau). \tag{9}$$

Thus, $\mathbf{G}(\tau)$ is a diagonal matrix with diagonals $\exp[\lambda(m)\tau]$.

The element form of Eq. 8 is

$$p(i, j, \tau) = \sum_{m=1}^{n} c(i, m)r(m, j)\exp[\lambda(m)\tau]. \tag{10}$$

The steady-state probability of being in a kinetic state i, $\phi(i)$, is equal to the probability that the channel is in state i at a very long time τ, given the channel is in state i at time 0 (Karlin and Taylor, 1975; Colquhoun and Hawkes, 1977):

$$\phi(i) = \lim_{\tau \to \infty} p(i, i, \tau). \tag{11}$$

Then

$$\phi(i) = c(i, n)r(n, i) = r(n, i). \tag{12}$$

PART II: KINETIC ANALYSIS OF STATIONARY PATCH-CLAMP DATA

A. Dwell Time Analyses

All of the Kinetic Information of Stationary Patch-Clamp Data Is Contained in Two-Dimensional Dwell Time Histograms. The following proof extends the work of Fredkin et al. (1985) and establishes that two-dimensional dwell time densities of patch-clamp data with x conductances have all of the kinetic information present in patch-clamp data. We begin with introductory material to establish mathematical concepts with regard to dwell times (for background see Colquhoun and Hawkes 1977, 1981, 1982, 1987; Fredkin et al., 1985; Fredkin and Rice, 1986).

Patch-clamp data report the current going through a channel at a given moment. Thus, the conductances of the different kinetic states determine the distinguishability of kinetic states. Let $B(I)$ be the subset of kinetic states having a common conductance $b(I)$, with the conductance subset identified by the designated conductance index I. Let N_I be the number of kinetic states belonging to $B(I)$. For x conductances there are x conductance subsets. The phrase "conductance I" refers to the subset $B(I)$ of kinetic states having conductance $b(I)$. A set of patch-clamp data can be described as a series of events during which a channel is in a given conductance I [has conductance $b(I)$] for a time τ_1, then in a conductance J for a time τ_2, etc. The τ terms are the dwell times of being in various conductances. To describe the probability of a sequence of such events, the rate constant matrix **Q** is partitioned into submatrices, $\mathbf{Q}_{IJ}$, each of which are of size N_I by N_J, with elements $q_{IJ}(i', j')$ (Colquhoun and Hawkes, 1977). Then,

$$q_{IJ}(i', j') = q(i, j) \quad \begin{array}{l} \text{for } i \in B(I),\ j \in B(J), \\ i' = 1 \text{ to } N_I,\ j' = 1 \text{ to } N_J \\ \text{for } I = 1 \text{ to } x \text{ and } J = 1 \text{ to } x, \end{array} \tag{13}$$

with $i \in B(I)$ meaning kinetic states i belonging to the subset of kinetic states $B(I)$. The indices i and i' refer to different enumerations to different matrices, but they match up to the same kinetic state. Henceforth, upper case index variables (I, J, etc.) refer to the conductance subsets, and lower case index variables (i, j, etc.) refer to the kinetic states.

Some relationships will now be established which will be used later in the proof. First, Eq. 1 can be arranged in matrix algebra form by

$$\mathbf{Qu} = \mathbf{0}, \tag{14}$$

where $\mathbf{u}$ is an n by 1 column vector of ones and $\mathbf{0}$ is an n by 1 column vector of zeroes. Since $\mathbf{Q}_{IJ}$ are partition matrices of $\mathbf{Q}$ in accordance with Eq. 13, Eq. 14 is equivalent to

$$\sum_{\substack{J=1 \\ J \neq I}}^{x} \mathbf{Q}_{IJ}\mathbf{u}_J = \mathbf{0}_I \quad \text{for } I = 1 \text{ to } x, \tag{15}$$

where $\mathbf{u}_I$ are N_I by 1 column vectors of ones and $\mathbf{0}_I$ is an N_I by 1 column vector of zeroes. Its transpose, $\mathbf{u}_I'$, is a N_I by 1 row vector of ones. There are n independent equations of 15, based on Eq. 1. Eq. 15 can be rearranged to

$$\sum_{\substack{J=1 \\ J \neq I}}^{x} \mathbf{Q}_{IJ}\mathbf{u}_J = -\mathbf{Q}_{II}\mathbf{u}_I \quad \text{for } I = 1 \text{ to } x. \tag{16}$$

In addition, we develop nomenclature for the steady-state probabilities of being in kinetic state i' of conductance I, by Eq. 12

$$\phi_I(i') = \phi(i) = r(n, i) \quad \begin{matrix} \text{for } i \in B(I) \\ i' = 1 \text{ to } N_I \\ I = 1 \text{ to } x. \end{matrix} \tag{17}$$

$\phi_I(i')$ and $q_{IJ}(i', j')$ are related by

$$\sum_{I=1}^{x}\sum_{i'=1}^{N_I} \phi_I(i')q_{IJ}(i', j') = \sum_{i=1}^{n} \phi(i)q(i, j) - \sum_{i=1}^{n}\sum_{p=1}^{n} r(n, i)c(i, p)\lambda(p)r(p, j) = 0 \tag{18}$$

by Eqs. 2, 4, 5, and 12. Let $\mathbf{\Phi}_I$ be an N_I by N_I diagonal matrix whose diagonals are $\phi_I(i')$ and let $\mathbf{\Phi}$ be an n by n diagonal matrix with elements $\phi(i)$. Then, Eq. 18 can be expressed in matrix form:

$$\sum_{I=1}^{x} \mathbf{u}_I'\mathbf{\Phi}_I\mathbf{Q}_{IJ} = \mathbf{0}_J', \tag{19}$$

where $\mathbf{u}'$ is a 1 by n row vector of ones and $\mathbf{0}_J'$ is a 1 by N_J row vector of zeroes. There are n equations of 19, but only $n - 1$ of these are linearly independent since $\mathbf{Q}$ has a rank of $n - 1$. Put another way, Eq. 19 establishes only the relative values of $\phi_I(i')$. The nth equation is the normalization of $\phi_I(i')$:

$$\sum_{I=1}^{x}\sum_{i'=1}^{N_I} \phi_I(i') = \sum_{i=1}^{n} \phi(i) = \sum_{i=1}^{n} r(n, i)c(i, n) = 1 \tag{20}$$

by Eqs. 17, 12, and 4. In matrix form,

$$\sum_{I=1}^{x} \mathbf{u}_I'\mathbf{\Phi}_I\mathbf{u}_I = 1. \tag{21}$$

In summary, Eqs. 16, 19, and 21 provide $2n$ independent equations that establish relationships among two-dimensional dwell time density amplitudes defined below (Eq. 25).

The $\mathbf{Q}_{II}$ matrices are square and can be diagonalized in the same manner as the $\mathbf{Q}$ matrix, with eigenvector matrix $\mathbf{C}_I$ and diagonal matrix Λ_I:

$$\mathbf{Q}_{II} = \mathbf{C}_I\Lambda_I\mathbf{R}_I \tag{22}$$

with elements $c_I(i, m)$ for $\mathbf{C}_I$, $r_I(m, j)$ for $\mathbf{R}_I$, and $\lambda_I(m)$ for diagonal elements of Λ_I. We stipulate that $\mathbf{Q}_{II}$ has N_I nonzero, distinct eigenvalues. The matrices $\mathbf{C}$ and $\mathbf{R}$ are inverses of each other and commute:

$$\mathbf{C}_I\mathbf{R}_I = I_d \tag{23}$$

or

$$\mathbf{R}_I\mathbf{C}_I = I_d. \tag{24}$$

With the nomenclature and relationships established, we construct the two-dimensional dwell time density, $P_2(\tau_1, \tau_2)$, as the probability density that the channel is in conductance I at $t = 0$, stays in conductance I for a time τ_1, changes to a different conductance J at some time between $t = \tau_1$ and $t = \tau_1 + d\tau_1$, stays in conductance J for a time τ_2, and changes to some other conductance at some time between $t = \tau_1 + \tau_2$ and $t = \tau_1 + \tau_2 + d\tau_2$ (Fredkin et al., 1985; Colquhoun and Hawkes, 1987)

$$\begin{aligned} P_2(\tau_1, \tau_2) = \frac{\partial f(\tau_1, \tau_2)}{\partial\tau_1\partial\tau_2} &= \mathbf{u}_I'\mathbf{\Phi}_I \exp(\mathbf{Q}_{II}\tau_1)\mathbf{Q}_{IJ} \\ &\cdot \exp(\mathbf{Q}_{JJ}\tau_2)\left(\sum_{\substack{K=1 \\ K \neq J}}^{x} \mathbf{Q}_{JK}\mathbf{u}_K\right) = (\mathbf{u}_I'\mathbf{\Phi}_I\mathbf{C}_I) \\ &\cdot \exp(\Lambda_I\tau_1)(\mathbf{R}_I\mathbf{Q}_{IJ}\mathbf{C}_J) \\ &\cdot \exp(\Lambda_J\tau_2)\left(\sum_{\substack{K=1 \\ K \neq J}}^{x} \mathbf{R}_J\mathbf{Q}_{JK}\mathbf{u}_K\right) \\ &= \mathbf{u}_I'\mathbf{S}_I\mathbf{G}_I(\tau_1)\mathbf{X}_{IJ}\mathbf{G}_J(\tau_2)\mathbf{T}_J\mathbf{u}_J \end{aligned} \tag{25}$$

with the following definitions: $\mathbf{S}_I$ is an N_I by N_I diagonal matrix with diagonal elements

$$s_I(r) = \sum_{i=1}^{N_I} \phi_I(i)c_I(i, r). \tag{26}$$

Thus,

$$\mathbf{u}_I'\mathbf{S}_I = \mathbf{u}_I'\mathbf{\Phi}_I\mathbf{C}_I. \tag{27}$$

Note that since $\mathbf{u}_I'$ is not invertible, we cannot assume $\mathbf{S}_I = \mathbf{\Phi}_I\mathbf{C}_I$.

$\mathbf{G}_I(\tau)$ is an N_I by N_I diagonal matrix with diagonal elements

$$g_I(m) = \exp[\lambda_I(m)\tau]. \tag{28}$$

$\mathbf{X}_{IJ}$ is an N_I by N_J matrix with elements

$$x_{IJ}(m1, m2) = \sum_{i=1}^{N_I}\sum_{j=1}^{N_J} r_I(m1, i)q_{IJ}(i, j)c_J(j, m2). \tag{29}$$

In matrix form

$$\mathbf{X}_{IJ} = \mathbf{R}_I\mathbf{Q}_{IJ}\mathbf{C}_J. \tag{30}$$

$\mathbf{T}_I$ is an N_I by N_I diagonal matrix with diagonal elements

$$t_I(m) = \sum_{i=1}^{N_I}\sum_{\substack{J=1\\J\neq I}}^{x}\sum_{j=1}^{N_J} r_I(m, i)q_{IJ}(i, j). \tag{31}$$

Thus,

$$\mathbf{T}_I\mathbf{u}_I = \mathbf{R}_I\left(\sum_{\substack{J=1\\J\neq I}}^{x}\mathbf{Q}_{IJ}\mathbf{u}_J\right). \tag{32}$$

The row vector $\mathbf{u}_I'$ multiplied by a diagonal matrix transfers the N_I diagonal elements to the N_I element positions of a row vector. For example, multiplying $\mathbf{u}_I'$ by $\mathbf{S}_I$ converts the diagonal matrix $\mathbf{S}_I$ into a row vector without loss of information. This is also true of diagonal matrices multiplied by $\mathbf{u}_I$.

While the S, G, X, and T matrices are derived as mathematical constructs, understanding their roles in the probability density function can be facilitated by thinking of them in terms of their physical significance. Thus, S is the steady-state probability of each "eigenvector state," G is the exponential decay of each eigenvector as a function of time, X is the probability of transition from the "eigenvector states" of I to states of J, and T is the probability of transition out of each "eigenvector state."

Because G, S, and T are diagonal matrices, their order of multiplication can be rearranged. Thus the probability Eq. 25 can be rearranged to group all of the components of the amplitudes to the exponentials (S, X, and T) into one term:

$$P_2(\tau_1, \tau_2) = \mathbf{u}_I'\mathbf{G}_I(\tau_1)(\mathbf{S}_I\mathbf{X}_{IJ}\mathbf{T}_J)\mathbf{G}_J(\tau_2)\mathbf{u}_J = \mathbf{u}_I'\mathbf{G}_I(\tau_1)\mathbf{A}_{IJ}\mathbf{G}_J(\tau_2)\mathbf{u}_J, \tag{33}$$

where

$$\mathbf{A}_{IJ} = \mathbf{S}_I\mathbf{X}_{IJ}\mathbf{T}_J \tag{34}$$

so the elements of $\mathbf{A}_{IJ}$ are

$$a_{IJ}(m1, m2) = s_I(m1)x_{IJ}(m1, m2)t_J(m2) \tag{35}$$

and are the amplitudes to the two-dimensional dwell time density.

Further, $\mathbf{T}_I\mathbf{u}_I$ can be simplified to

$$\mathbf{T}_I\mathbf{u}_I = -\mathbf{R}_I\mathbf{Q}_{II}\mathbf{u}_I = -\mathbf{\Lambda}_I\mathbf{R}_I\mathbf{u}_I \tag{36}$$

by Eqs. 32, 16, 22, and 24.

The amplitudes $a_{IJ}(m1, m2)$ and the eigenvalues $\lambda_I(m1)$ represent the data, for $m1 = 1$ to N_I, $m2 = 1$ to N_J, for all I and J, $I \neq J$. The term $a_{II}(m1, m2)$ is not present in the data, but we shall evaluate the corresponding $x_{II}(r1, r2)$ for future use:

$$\mathbf{X}_{II} = \mathbf{R}_I\mathbf{Q}_{II}\mathbf{C}_I = \mathbf{\Lambda}_I \tag{37}$$

by Eqs. 30, 22, and 24.

To prove that two-dimensional dwell time densities have all of the information of stationary patch-clamp data, we first show the relationship between A and T.

$$\begin{aligned}\sum_{\substack{J=1\\J\neq I}}^{x} -\mathbf{A}_{IJ}\mathbf{A}_J^{-1}\mathbf{u}_J &= -\sum_{\substack{J=1\\J\neq I}}^{x}\mathbf{S}_I\mathbf{X}_{IJ}\mathbf{T}_J\mathbf{A}_J^{-1}\mathbf{u}_J\\ &= -\sum_{\substack{J=1\\J\neq I}}^{x}\mathbf{S}_I\mathbf{X}_{IJ}\mathbf{A}_J^{-1}\mathbf{T}_J\mathbf{u}_J\\ &= \sum_{\substack{J=1\\J\neq I}}^{x}\mathbf{S}_I\mathbf{R}_I\mathbf{Q}_{IJ}\mathbf{C}_J\mathbf{A}_J^{-1}\mathbf{\Lambda}_J\mathbf{R}_J\mathbf{u}_J\\ &= \mathbf{S}_I\left(\sum_{\substack{J=1\\J\neq I}}^{x}\mathbf{R}_I\mathbf{Q}_{IJ}\mathbf{u}_J\right) = \mathbf{S}_I\mathbf{T}_I\mathbf{u}_I\end{aligned} \tag{38}$$

by Eqs. 34, 30, 36, 23, and 32. Note that the n independent equations of 38 are established because of Eq. 36, which in turn are true by the n independent equations of 16, which in turn are based on the n independent equations 1. We define the diagonal matrix

$$\mathbf{V}_I = \mathbf{S}_I\mathbf{T}_I. \tag{39}$$

So,

$$\mathbf{V}_I^{-1} = \mathbf{T}_I^{-1}\mathbf{S}_I^{-1}. \tag{40}$$

Since Eq. 38 represents the integration of Eq. 25 over all τ_2, summed over all J, $J \neq I$, the elements of $\mathbf{V}_I$ are the amplitudes to the one-dimensional density:

$$\begin{aligned}\sum_{\substack{J=1\\J\neq I}}^{x}\int_0^\infty P_2(\tau_1, \tau_2)\,d\tau_2 &= \sum_{\substack{J=1\\J\neq I}}^{x}\mathbf{u}_I'\mathbf{\Phi}_I\exp(\mathbf{Q}_{II}\tau_1)\mathbf{Q}_{IJ}\\ &\quad\cdot\left\{\int_0^\infty \exp[\mathbf{Q}_{JJ}(\tau_2)(-\mathbf{Q}_{JJ})\,d\tau_2]\right\}\mathbf{u}_J\\ &= \mathbf{u}_I'\mathbf{V}_I\mathbf{G}_I(\tau_1)\mathbf{u}_I = P_1(\tau_1).\end{aligned} \tag{40a}$$

By extension of the procedure for expressing the probability of occurrence for a sequence of two dwell times, an m

dimensional dwell time density is

$$P_m[I(1), \tau_1, I(2), \tau_2, \ldots, I(m), \tau_m] = \mathbf{u}'_{I(1)}\mathbf{S}_{I(1)}\mathbf{G}_{I(1)}(\tau_1) \cdot \left[\prod_{p=1}^{m-1} \mathbf{X}_{I(p),I(p+1)}\mathbf{G}_{I(p+1)}(\tau_{p+1})\right]\mathbf{T}_{I(m)}\mathbf{u}_{I(m)} = \mathbf{u}'_{I(1)}\mathbf{G}_{I(1)}(\tau_1) \cdot \left\{\prod_{p=1}^{m-2} [\mathbf{S}_{I(p)}\mathbf{X}_{I(p),I(p+1)}\mathbf{T}_{I(p+1)}][\mathbf{T}^{-1}_{I(p+1)}\mathbf{S}^{-1}_{I(p+1)}]\mathbf{G}_{I(p+1)}(\tau_{p+1})\right\} \cdot [\mathbf{S}_{I(m-1)}\mathbf{X}_{I(m-1),I(m)}\mathbf{T}_{I(m)}]\,\mathbf{G}_{I(m)}(\tau_m)\mathbf{u}_{I(m)} = \mathbf{u}'_{I(1)}\mathbf{G}_{I(1)}(\tau_1) \cdot \left[\prod_{p=1}^{m-2} \mathbf{A}_{I(p),I(p+1)}\mathbf{V}^{-1}_{I(p+1)}\,\mathbf{G}_{I(p+1)}(\tau_{p+1})\right] \cdot \mathbf{A}_{I(m-1),I(m)}\mathbf{G}_{I(m)}(\tau_m)\mathbf{u}_{I(m)} \quad (41)$$

by Eqs. 34 and 40, where $I(p)$ is the conductance index corresponding to the conductance $b(I)$ of the channel at the pth sequential dwell time, τ_p. Note that S, T, G, and their inverses are diagonal matrices, so their order of multiplication can be interchanged. Also recall that $\mathbf{V}_I$ is a construct that is a function of the data amplitudes and the eigenvalues of the two-dimensional dwell time densities by Eqs. 38 and 39. Thus, Eq. 41 expresses any m dimensional dwell time density as a function of the amplitudes and eigenvalues of a two-dimensional dwell time density. If there are m dwell times in a data set, the m dimensional dwell time density describes the probabilistic occurrence of all of the events in that data set exactly as they occurred and has all of the kinetic information of the original data (cf. Horn and Vandenberg, 1984). Thus, the two-dimensional dwell time density has all of the kinetic information in patch-clamp data.

The Maximum Number of Rate Constants That Can Be Determined from Stationary Patch-Clamp Data. To determine the number of independent rate constants that can be calculated, we note there is at least the following interdependence among the data amplitudes:

$$\sum_{I=1}^{x} \mathbf{u}'_I\mathbf{A}_{IJ} = \sum_{I=1}^{x} \mathbf{u}'_I\mathbf{S}_I\mathbf{X}_{IJ}\mathbf{T}_J - \sum_{I=1}^{x} \mathbf{u}'_I\mathbf{\Phi}_I\mathbf{C}_I\mathbf{R}_I\mathbf{Q}_{IJ}\mathbf{C}_J\mathbf{T}_J - \left(\sum_{I=1}^{x} \mathbf{u}'_I\mathbf{\Phi}_I\mathbf{Q}_{IJ}\right)\mathbf{C}_J\mathbf{T}_J = \mathbf{0}_J \quad (42)$$

by Eqs. 35, 30, 27, and 19. Eq. 42 can be rearranged (by Eqs. 42, 35, 37, and 39) to give

$$\sum_{\substack{I=1 \\ I\neq J}}^{x} \mathbf{u}'_I\mathbf{A}_{IJ} = -\mathbf{u}'_J\mathbf{A}_{JJ} = -\mathbf{u}'_J\mathbf{S}_J\mathbf{X}_{JJ}\mathbf{T}_J = -\mathbf{u}'_J\mathbf{S}_J\mathbf{\Lambda}_J\mathbf{T}_J = -\mathbf{u}'_J\mathbf{S}_J\mathbf{T}_J\mathbf{\Lambda}_J = -\mathbf{u}'_J\mathbf{V}_J\mathbf{\Lambda}_J. \quad (43)$$

Eqs. 43 are true because of the $n - 1$ independent equations of 19. Thus, as with Eqs. 19, there are only $n - 1$ independent equations of 43. The equations are normalized by the nth equation

$$-\sum_{I=1}^{x} \mathbf{u}'_I\mathbf{V}_I\mathbf{\Lambda}_I^{-1}\mathbf{u}_I = -\sum_{I=1}^{x} \mathbf{u}'_I\mathbf{S}_I\mathbf{\Lambda}_I^{-1}\mathbf{T}_I\mathbf{u}_I = \sum_{I=1}^{x} \mathbf{u}'_I\mathbf{\Phi}_I\mathbf{u}_I = 1, \quad (44)$$

obtained by combining Eqs. 39, 36, 27, 17, and 21. In summary, Eqs. 43 and 44 together make up n independent equations (or N_J for each J), based on the n independent equations of 19 and 21, and thus place n constraints on Eqs. 35 pertaining to the data amplitudes.

Eq. 44 represents the integration of Eqs. 40a over all τ_1, and summation over all I:

$$\sum_{I=1}^{x} \int_0^\infty \mathbf{u}'_I\mathbf{V}_I\mathbf{G}_I(\tau_1)\mathbf{u}_I\,d\tau_1 = \sum_{I=1}^{x} \mathbf{u}'_I\mathbf{\Phi}_I\left[\int_0^\infty \exp(\mathbf{Q}_{II}\tau_1)(-\mathbf{Q}_{II})\,d\tau_1\right]\mathbf{u}_I = \sum_{I=1}^{x} \mathbf{u}'_I\mathbf{\Phi}_I\mathbf{u}_I = 1. \quad (44a)$$

Thus, the two-dimensional and one-dimensional probability densities normalize to unity.

Counting up the amplitudes, we have for $m1 = 1$ to N_I, $m2 = 1$ to N_J, for all I and J, except for I equal to J,

$$\sum_{I=1}^{x} \sum_{\substack{J=1 \\ J\neq I}}^{x} N_I N_J = 2\left[\sum_{I=1}^{x-1} \sum_{J=I+1}^{x} N_I N_J\right] = H. \quad (45)$$

But only $H - n$ of these amplitudes are independent, since Eqs. 43 and 44 place n constraints upon them. However, there are n total λ_I eigenvalues, giving a maximum of H independent parameters among the data amplitudes and eigenvalues for the evaluation of the rate constants. Thus, at most H independent rate constants can be calculated from stationary patch-clamp data for channels with x conductances. Note that for $x = 2$, Eq. 45 reduces to $H = 2N_oN_c$ where N_o is the number of open states and N_c is the number of closed states (Fredkin et al., 1985).

Calculating the Maximum Number of Rate Constants from Stationary Patch-Clamp Data. We have shown that stationary patch-clamp data have information to determine not more than H rate constants. Accordingly, to calculate these rate constants, at least

$$G = n(n-1) - H = \sum_{I=1}^{x} N_I(N_I - 1) \quad (46)$$

rate constants must be fixed. We now consider the conditions under which the maximum number of H rate constants can be calculated from patch-clamp data.

We note that if $x = n$, then $H = n(n-1)$. That is, if the occupancy of each kinetic state can be directly observed, patch-clamp data have information to completely describe a kinetic model of channel gating. In addition, we point out that there are G intraconductance rate constants [i.e., rate

constants $q(i,j)$ where i and $j \in B(I)$]. That is, the number of rate constants that must be fixed is equal to the number of transitions that cannot be directly observed in patch-clamp data. It has been our experience that if we choose to fix the intraconductance rate constants, the remaining H rate constants are specified, that is, can be calculated with no remaining degrees of freedom. However, if G rate constants are fixed which are not all intraconductance rate constants, then often but not always, the remaining H rate constants are specified. Some examples are given in a subsequent section.

In this regard, we note that the intraconductance rate constants are the nondiagonal elements to the $\mathbf{Q}_{II}$ matrices, for each I. The diagonal elements $q_{II}(i,i)$ may not be fixed since they are dependent on the other rate constants by Eq. 15. However, we know the eigenvalues to the $\mathbf{Q}_{II}$ matrix, $\mathbf{\Lambda}_I$, as the exponential constants to dwell time densities. By definition, eigenvalues satisfy the following equations:

$$\det[\mathbf{Q}_{II} - \lambda_I(m)\mathbf{I}_d] = 0 \quad \text{for } m = 1 \text{ to } N_I, \tag{47}$$

where det[] means determinant of the expression in brackets, and $\mathbf{I}_d$ is the identity matrix. Eqs. 47 yield N_I equations with N_I unknowns $q_{II}(i,i)$. The solution for the diagonal elements via Eq. 47 is nonlinear, and we are not aware of a mathematical proof stating that from the N_I equations of 47 the $q_{II}(i,i)$ diagonal elements are specified. However, we have solved for the $q_{II}(i,i)$ analytically for 2 by 2 and 3 by 3 matrices and found the postulate to be true. Accordingly, we postulate that for any N_I size $\mathbf{Q}_{II}$ matrix the diagonal elements can be specified from a set of nondiagonal $q_{II}(i,j)$ elements and the known eigenvalues.

If the $\mathbf{Q}_{II}$ matrix is specified, then $\mathbf{C}_I$ and its inverse can be evaluated (Eq. 22). Then, $\mathbf{T}_I$ can be evaluated by Eq. 36. $\mathbf{S}_I$ can then be evaluated by Eq. 38, using the experimental values of $\mathbf{A}_{IJ}$ and $\mathbf{\Lambda}_I$, and the calculated $\mathbf{T}_I$. Then $\mathbf{X}_{IJ}$ can be evaluated by Eq. 34, and the H rate constants represented by $\mathbf{Q}_{IJ}$ can be calculated by

$$\mathbf{Q}_{IJ} = \mathbf{C}_I\mathbf{X}_{IJ}\mathbf{R}_J. \tag{48}$$

Note that neither uniqueness nor existence of at least one real valued solution is guaranteed for any set of G fixed rate constants. For example, for a 2 × 2 matrix, the unknown $q_{II}(i,i)$ elements are the roots to a polynomial equation derived from Eq. 47, but the equations do not specify which element of $q_{II}(i,i)$ has a value of which root. Thus, two answers for $\mathbf{Q}_{II}$ are possible for one set of G intraconductance assignments. Similarly, if at least one pair of $q_{II}(i,i)$ is complex, then no real valued solution exists for the G assignments. If the intraconductance rate constants within a conductance are all assigned the same value, the solutions will be indistinguishable as kinetic models, so that if there is a solution, there will be only one solution.

The assignment of all intraconductance rate constants to zero deserves attention. Then each $\mathbf{Q}_{II}$ is a diagonal matrix, so

$$\mathbf{Q}_{II} = \mathbf{C}_I\mathbf{\Lambda}_I\mathbf{R}_I = \mathbf{\Lambda}_I \tag{49a}$$

$$\mathbf{C}_I = \mathbf{I}_d. \tag{49b}$$

That is, the empirically derived eigenvalues of $\mathbf{\Lambda}_I$ are the diagonals of $\mathbf{Q}_{II}$, and since all of the nondiagonal values of $\mathbf{Q}_{II}$ have the same value of zero, a unique solution always exists. However, a solution using assignments that result in negative values for some rate constants is not a solution to the kinetics problem.

In summary, we postulate that for an appropriately chosen set of G intraconductance rate constants, H rate constants can be specified from patch-clamp data. Thus, a countable finite number of solutions to the H rate constants can be calculated, not an infinite number of solutions.

B. Point Joint Probability Analyses

The Point Joint Probability Method of Analysis. The previous section demonstrates that analysis of dwell times is useful for the determination of the number of rate constants that can be determined from stationary patch-clamp data. We now consider an alternative approach: the analysis of patch-clamp data via point joint probabilities. The major advantage of this approach is that in the analysis of data with more than one channel, the exponential terms do not combine in a multinomial manner, as is the case with dwell time methods of analysis (Bauer, R.J., manuscript in preparation). In addition, for the calculation of rate constants, only one matrix $\mathbf{Q}$ and eigenvector system $\mathbf{C}$ must be dealt with in the numerical analysis. This provides for the rapid calculation of rate constants from a data set and different sets of rate constant assignments.

The point joint probability function is based on the conditional probability, $p(i,j,\tau)$, and the steady-state probability, $\phi(i)$, described earlier. We define the two-point joint probability as the unconditional probability that a channel is in some state i at some moment, and in state j a time τ later. That is,

$$\phi(i)p(i,j,\tau). \tag{50}$$

This is the joint probability of a channel being in kinetic state i and j at two points in a time τ apart. It differs from the dwell time density in that it does not consider what happened during τ or how long a channel is in state i.

By extension, the three-point joint probability is defined as the probability that a channel is in state i, then in state j a time τ_1 later, and in state k a time τ_2 thereafter. That is, by Eqs. 10 and 50,

$$\phi(i)p(i,j,\tau_1)p(j,k,\tau_2) = \sum_{m1=1}^{n}\sum_{m2=1}^{n} r(n,i)c(i,m1)$$
$$r(m1,j)c(j,m2)r(m2,k) \cdot \exp[\lambda(m1)\tau_1 + \lambda(m2)\tau_2]. \tag{51}$$

Single channel data report the conductance level of the channel at any moment, not its kinetic state. That is, the data report the joint probability of a channel having conductance $b(I)$ at time 0, having conductance $b(J)$ at time τ_1, etc. Eq. 51 can be rewritten in terms of conductances by summing over all the kinetic states showing a given conductance level. For, example, the three-point joint conductance probability is

$$\sum_{i\in B(I)}\sum_{j\in B(J)}\sum_{k\in B(K)}\phi(i)p(i,j,\tau_1)\cdot p(j,k,\tau_2) = \sum_{i\in B(I)}\sum_{j\in B(J)}\sum_{k\in B(K)}\cdot\sum_{m1=1}^{n}\sum_{m2=1}^{n} r(n,i)c(i,m1)r(m1,j)\cdot c(j,m2)r(m2,k)\cdot \exp[\lambda(m1)\tau_1+\lambda(m2)\tau_2]. \quad (52)$$

This in turn is equal to

$$\sum_{m1=1}^{n}\sum_{m2=1}^{n}\left[\sum_{i\in B(I)}(n,i)c(i,m1)\right]\cdot\left[\sum_{j\in B(J)} r(m1,j)c(j,m2)\right]\cdot\left[\sum_{k\in B(K)} r(m2,k)c(k,n)\right]\cdot \exp[\lambda(m1)\tau_1+\lambda(m2)\tau_2]. \quad (53)$$

[The inclusion of term $c(k, n)$ does not change the value of Eq. 53, since $c(k, n) = 1$]. The arrangement of the terms in brackets in Eq. 53 suggests a convenient mathematical construct, the w_I construct:

$$w_I(m,p) = \sum_{i\in B(I)} r(m,i)c(i,p) \quad (54)$$

and the three-point joint conductance probability (Eq. 53) can now be written as

$$P_3(\tau_1,\tau_2) = \sum_{m1}^{n}\sum_{m2}^{n} w_I(n,m1)w_J(m1,m2)\cdot w_K(m2,n)\exp[\lambda(m1)\tau_1+\lambda(m2)\tau_2]. \quad (55)$$

Eq. 55 can be put into matrix notation by defining the matrix $\mathbf{W}_I$ which contains $w_I(m1, m2)$ and the diagonal matrices $\mathbf{W}_{n,I}$ and $\mathbf{W}_{I,n}$ whose diagonal elements are

$$w_{n,I}(m) = w_I(n,m) \quad (57)$$

$$w_{I,n}(m) = w_I(m,n). \quad (58)$$

Eq. 55 can be written as

$$P_3(\tau_1,\tau_2) = \mathbf{u}'\mathbf{W}_{n,I}\mathbf{G}(\tau_1)\mathbf{W}_J\mathbf{G}(\tau_2)\mathbf{W}_{K,n}\mathbf{u} = \mathbf{u}'\mathbf{G}(\tau_1)[\mathbf{W}_{n,I}\mathbf{W}_J\mathbf{W}_{K,n}]\mathbf{G}(\tau_2)\mathbf{u} = \mathbf{u}'\mathbf{G}(\tau_1)\mathbf{A}_{IJK}\mathbf{G}(\tau_{12})\mathbf{u}, \quad (59a)$$

where $\mathbf{u}'$ is a 1 by n row vector of ones and $\mathbf{A}_{IJK}$ is the matrix of three point probability amplitudes:

$$\mathbf{A}_{IJK} = \mathbf{W}_{n,I}\mathbf{W}_J\mathbf{W}_{K,n}. \quad (59b)$$

Similarly, an m point joint probability is

$$P_m(\tau_1,\tau_2,\ldots,\tau_{m-1}) = \mathbf{u}'\mathbf{W}_{n,I(1)}\cdot\left[\prod_{p=1}^{m-2}\mathbf{G}(\tau_p)\mathbf{W}_{I(p+1)}\right]\mathbf{G}(\tau_{m-1})\mathbf{W}_{I(m),n}\mathbf{u}, \quad (60)$$

where $I(p)$ is the conductance index pertaining to conductance $b(I)$ of the channel at the pth point and τ_p is the time between the pth and $(p + 1)$th points. We point out that the W constructs were created as a mathematical convenience; their physical significance, if any, is unclear.

Like the m dimensional dwell time density, the m-point joint probability distribution is a multiple exponential function with amplitudes determined by the elements of an eigenvector matrix and time constants determined by the elements of an eigenvalue matrix. Also like the m dimensional dwell time density, the m-point joint probability can describe the probabilistic occurrence of all of the events in a digitized data record. That is, for a data set of m digital data points describing the sequence of events, each point representing the constant sampling interval of $\delta\tau$, a probability equation that describes the full sequence of events in that data set is an m-point joint probability equation for which $\tau_1 = \tau_2 = \tau_3 = \ldots\ldots = \tau_{m-1} = \delta\tau$. Because such an m-point joint probability considers all of the events in a data set exactly as they happened (i.e., there is no averaging) this function contains all of the kinetic information in the data. Eq. 60 demonstrates that this information is found in the $n - 1$ nonzero eigenvalues plus the $\mathbf{W}_I$ constructs.

We now show that the three-point joint probability contains all of the information of the m-point joint probability, and hence contains all of the information present in stationary patch-clamp data. Note that some of the W constructs are related to each other. For each column eigenvector, one of the eigenvector elements may be set to an arbitrary value. For the nth column, we retain the designation from Eq. 7 of $c(j,n) = 1$, for $j = 1$ to n. For columns $m = 1$ to $n - 1$, it is convenient to specify one of the W constructs of a specific conductance, $I = S$, for each column vector m, such that

$$w_S(n,m) = w_S(n,n) \qquad m = 1 \text{ to } n-1. \quad (61)$$

This is to say that

$$\sum_{i\in B(S)} r(n,i)c(i,m) = \sum_{i\in B(S)} r(n,i)c(i,n). \quad (62)$$

If conductance S has only one kinetic state, this reduces to the familiar designation of $c(i,m) = 1$ for the single kinetic state i of conductance S.

Next, we note that $\mathbf{W}_I$ can be expressed as

$$\mathbf{W}_I = \mathbf{R}\boldsymbol{\Gamma}_I\mathbf{C}, \tag{63}$$

where $\boldsymbol{\Gamma}_I$ is a diagonal matrix with diagonal elements

$$\boldsymbol{\Gamma}_I(i,i) = \delta(i,I), \tag{64a}$$

where

$$\delta(i,I) = 0 \text{ for } i \notin B(I)$$
$$= 1 \text{ for } i \in B(I).$$

Since i can belong to one and only one conductance subset $B(I)$,

$$\boldsymbol{\Gamma}_I\boldsymbol{\Gamma}_J = \boldsymbol{\Gamma}_I\delta(I,J) \tag{64b}$$

where

$$\delta(I,J) = 1 \quad \text{if } I = J$$
$$\delta(I,J) = 0 \quad \text{if } I \neq J$$

and

$$\sum_{I=1}^{x}\boldsymbol{\Gamma}_I = \mathbf{I}_d. \tag{64c}$$

Some relationships that will be useful are

$$\mathbf{W}_I\mathbf{W}_J = \mathbf{W}_I\delta(I,J) \tag{65a}$$

and

$$\sum_{I=1}^{x}\mathbf{W}_I = \mathbf{I}_d \tag{65b}$$

from Eqs. 63 and 64, showing that if the $\mathbf{W}$ constructs for $x-1$ of the conductances are known, the $\mathbf{W}$ constructs for the xth conductance can be calculated.

With these relationships established, we turn to the three-point joint probability. By Eq. 59b, the amplitudes of a three-point joint probability are

$$a_{IJK}(m1,m2) = w_I(n,m1)w_J(m1,m2)w_K(m2,n). \tag{66}$$

For $I = S$ and $K = J$,

$$\frac{a_{SJJ}(m1,m2)}{\sum_{p=1}^{n}a_{SJJ}(m2,p)} = \frac{w_S(n,m1)w_J(m1,m2)w_J(m2,n)}{\sum_{p=1}^{n}w_S(n,m2)w_J(m2,p)w_J(p,n)}$$
$$= \frac{w_S(n,m1)w_J(m1,m2)w_J(m2,n)}{w_S(n,m2)w_J(m2,n)} = w_J(m1,m2) \tag{67}$$

by Eqs. 61 and 65. Eq. 67 shows that each $\mathbf{W}$ construct can be calculated explicitly from the amplitudes of a three-point joint probability, i.e., the three-point joint probability function has all of the kinetic information of patch-clamp data.

By Eq. 67, from each S-J-J point joint probability the constructs of $\mathbf{W}_J$ can be calculated. We need only the S-J-J point joint probabilities for $x-1$ of the J's, since the xth $\mathbf{W}_J$ can be calculated from the $x-1$ other $\mathbf{W}_J$ by Eq. 65b. For an open-closed ($x = 2$ conductances) system, if the open conductance is designated as the S conductance, then the S-J-J point joint probability for which J = open (i.e., the open-open-open point joint probability) is sufficient to obtain all of the kinetic information available from the patch-clamp data.

It is of interest to consider another special case of a channel showing two conductances ($x = 2$, open and closed), where the open conductance has one kinetic state, with multiple closed states. In this case $N_o = 1, N_c = n-1$, where n is the total number of kinetic states. Then Eq. 45 yields

$$H = 2(n-1).$$

Thus, channels with only one open state can be readily analyzed with one-dimensional analytical methods, such as dwell time histograms or two-point joint probabilities (Bauer and Kenyon, 1987), since these analyses yield $2(n-1)$ independent parameters. More generally, if $x-1$ of the conductances have single kinetic states, while one of the conductances has one or more kinetic states, then a two-point joint probability analysis is sufficient to obtain all of the information in patch-clamp data.

Maximum Number of Rate Constants That Can Be Calculated as Derived from Three-Point Probability. The result embodied in Eqs. 45 and 46 can be readily derived when we consider the total patch-clamp data as expressed by the three-point joint probability amplitude component $\mathbf{W}_I$, and the eigenvalue matrix $\boldsymbol{\Lambda}$. Consider that $\mathbf{W}_I$ is equal to

$$\mathbf{W}_I = \mathbf{R}\boldsymbol{\Gamma}_I\mathbf{C}. \tag{63}$$

Since $\mathbf{Q}$ is diagonalizable, $\mathbf{C}$ and $\mathbf{R}$ both exist, and $\mathbf{W}_I$ is diagonalizable with eigenvalue matrix $\boldsymbol{\Gamma}_I$. The question is, to what degree does $\mathbf{W}_I$ specify the matrix $\mathbf{C}$ or $\mathbf{R}$ for the calculation of $\mathbf{Q}$?

We do not know what $\mathbf{Q}$ is; we only know $\mathbf{W}_I$ empirically. Thus, we want to know to what degree $\mathbf{R}$ can be known from $\mathbf{W}_I$, as the eigenvector matrix of $\mathbf{W}_I$. For each $\mathbf{W}_I$, N_I of the diagonal elements in the eigenvalue matrix $\boldsymbol{\Gamma}_I$ have a value of 1, and thus, N_I vectors $\mathbf{r}(i)$ have the same eigenvalue, for $i \in B(I)$. Since $\mathbf{W}_I$ is diagonalizable, there must be N_I linearly independent eigenvector columns for this eigenvalue. This requires that the use of matrix $\mathbf{W}_I$ to evaluate $\mathbf{r}(i)$ results in N_I degrees of freedom for each $\mathbf{r}(i)$, via the equation

$$[\mathbf{W}_I - \delta(i,I)\mathbf{I}_d]\mathbf{r}(i) = \mathbf{0}_I \qquad i \in B(I). \tag{68a}$$

Thus for each $\mathbf{W}_I$, there are N_IN_I degrees of freedom among the eigenvectors. For all $\mathbf{W}_I$, there is a total of

$$G' = \sum_{I=1}^{x}N_IN_I \tag{68b}$$

degrees of freedom, as calculated from all of the $\mathbf{W}_I$. In addition, the elements of $\mathbf{W}_I$ itself are dependent on the $n - 1$ arbitrary designations of Eq. 61. Thus, the number of independent parameters that can be calculated in $\mathbf{R}$ is

$$H' = n^2 - G' - (n - 1). \quad (68c)$$

To specifiy $\mathbf{Q}$, both the elements of $\mathbf{C}$ (or $\mathbf{R}$) plus the $n - 1$ nonzero eigenvalues of $\mathbf{\Lambda}$ are used, so the maximum number of independent parameters for calculating $\mathbf{Q}$ that can be obtained from the data, in the form of $\mathbf{W}_I$ and $\mathbf{\Lambda}$, is

$$H = H' + (n - 1) = n(n - 1) - \sum_{I=1}^{x} N_I(N_I - 1). \quad (69a)$$

C. Examples of the Kinetic Analysis of Stationary Patch-Clamp Data

We tested the hypothesis of Eq. 46 by making up arbitrary sets of rate constants, calculating the amplitudes and exponential constants of the point probability functions for the rate constant set, and using these "data" to attempt to calculate rate constants (see below). Note that in this approach, the "data" were calculated analytically to eight significant digits and represent a perfect sample. This procedure examines the ability of the method to recover rate constants from the selected models. It represents a best case analysis in the sense that real data will be noisy and will provide only an approximation of the true point probability functions.

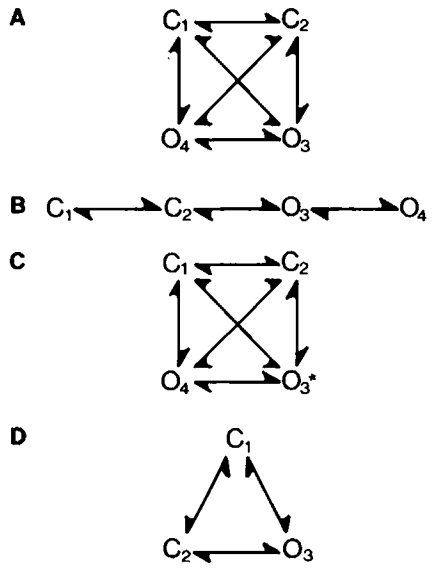

FIGURE 1 *A–C* are four state, two conductance kinetic schemes described in the text. Each state is identified by its conductance (C for closed, O for open) and an arbitrary number. The rate constants for the transitions are listed in Table I, where $q(1,2)$ refers to the rate of transition from state C_1 to state C_2, etc. In the figure, arrows are drawn for the transitions with rate constants greater than zero. Forbidden transitions are not shown. In *C* the two open states, O_3^* and O_4, have different conductances. Panel *D* is a three-state, two-conductance model discussed in the text.

Specifically, we made up a matrix $\mathbf{Q}$ of rate constants and then calculated the amplitudes $\mathbf{A}_{IJK}$ and exponential constants $\mathbf{\Lambda}$ to the three-point joint probability by diagonalizing the $\mathbf{Q}$ matrix using the *QR* factorization method (Isaacson and Keller, 1966). This yielded the eigenvalues (exponential constants). We calculated $\mathbf{C}$ and $\mathbf{R}$ matrices by linear algebraic methods and used these to calculate the amplitudes via Eq. 66. These amplitudes and exponential constants served as the "data," i.e., the point probability functions that would be generated by the model. As shown earlier, these amplitude and exponential constants to the three-point joint probabilities contain all of the kinetic information in stationary patch-clamp data. Rate constants were then calculated by fixing some number of rate constants and using these parameters, and the amplitudes and exponential constants of the point probability functions. A Gauss-Newton iterative procedure (Isaacson and Keller, 1966) was used to calculate the unknown eigenvector elements $c(i,m)$ from Eq. 66. The amplitudes $\mathbf{A}_{IJK}$ on the left-hand side were the empirical amplitudes. The equation

$$q(i,j) = \sum_{m=1}^{n} c(i,m)\lambda(m)r(m,j) \quad (69b)$$

was used for each $q(i,j)$ that was fixed. Thus, Eq. 69b for the assignments and Eq. 66 for the empirical amplitudes were the equations for the numerical analysis for finding the $c(i,m)$ and $r(m,j)$ for that particular set of data and assignments. Finally, when $\mathbf{C}$ and $\mathbf{R}$ were calculated in this manner, the unknown rate constants were calculated by Eq. 2. As discussed below, this procedure succeeded in calculating the unfixed rate constants with complete specificity only when G or more of the rate constants were fixed, as predicted by Eq. 46.

Fig. 1 *A* and Table I, column 1 illustrate and list the rate constants for one kinetic scheme that we examined. This model has two closed plus two open states and 12 nonzero

TABLE I
RATE CONSTANTS FOR THE KINETIC MODELS SHOWN IN FIG. 1

Rate constant	Column 1 Fig. 1, *A* and *C*	Column 2 Fig. 1 *B*	Column 3 Fig. 1 *A*
$q(1,2)$	1.0	1.0	1.0
$q(1,3)$	2.0	0.0	3.0
$q(1,4)$	3.0	0.0	3.0
$q(2,1)$	4.0	2.0	2.0
$q(2,3)$	5.0	3.0	4.0
$q(2,4)$	6.0	0.0	4.0
$q(3,1)$	7.0	0.0	5.0
$q(3,2)$	8.0	4.0	5.0
$q(3,4)$	9.0	5.0	1.0
$q(4,1)$	10.0	0.0	6.0
$q(4,2)$	11.0	0.0	6.0
$q(4,3)$	12.0	6.0	3.0

The rate constants are arbitrary units (time^{-1}).

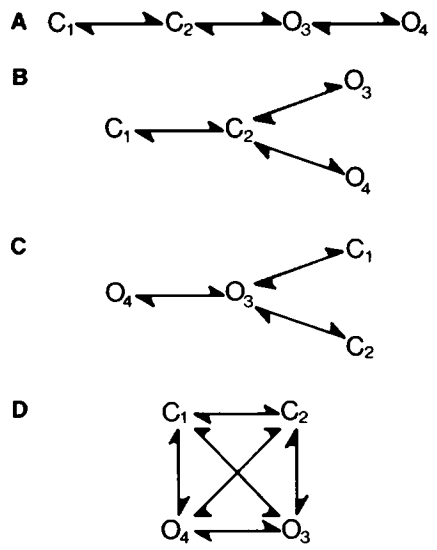

FIGURE 2 *A–D* are four-state, two-conductance kinetic models with different thermodynamic properties and branching that produce identical patch-clamp data with the rate constants listed in Table II. Conventions as in Fig. 1.

TABLE II
RATE CONSTANTS FOR KINETIC MODELS SHOWN IN FIG. 2

	Figure			
Rate constant	2 *A*	2 *B*	2 *C*	2 *D*
$q(1,2)$	1.0	1.0	0.0	0.010913845
$q(1,3)$	0.0	0.0	5.4494897	0.5
$q(1,4)$	0.0	0.0	0.0	0.043355212
$q(2,1)$	2.0	2.0	0.0	1.6859393
$q(2,3)$	3.0	2.4244734	0.55051027	3.4597916
$q(2,4)$	0.0	0.57552651	0.0	0.3
$q(3,1)$	0.0	0.0	2.0	1.0
$q(3,2)$	4.0	1.8210917	2.0	3.2333133
$q(3,4)$	5.0	0.0	5.0	7.3797926
$q(4,1)$	0.0	0.0	0.0	0.30928027
$q(4,2)$	0.0	13.178908	0.0	1.0
$q(4,3)$	6.0	0.0	6.0	2.0776136

The rate constants are arbitrary units (time^{-1}).

rate constants. We used the rate constants listed in Table I, column 1, to calculate the amplitudes and exponential constants of the three-point joint probability that the channel was open and "analyzed" these "data." After fixing the four intraconductance rate constants by assigning them their "correct" values, i.e., the values used to generate the data, the remaining eight rate constants could be calculated, consistent with Eq. 45 and 46 above. In addition, we found many sets of four rate constant assignments, that were not limited to the intraconductance rate constants, that also enabled the calculation of eight rate constants. For example, if we fixed $q(1,4)$, $q(4,1)$, $q(1,3)$, and $q(3,1)$ to the values of Table I, column 1, this resulted in the specification of the remaining eight rate constants. However, for some other rate constant assignments, the numerical analysis did not find a solution that fit Eqs. 66 and 69b. This may have been because some rate constant assignments yield complex valued solutions and our numerical procedures were limited to the calculation of real rate constants.

We also considered a system with two open states plus two closed states where six of the rate constants are zero (Fig. 1 *B*, and Table I, column 2). Again, the assignment of the intraconductance rate constants to their correct values enabled calculation of the remaining eight interconductance rate constants.

In these examples, the determination of $H = 8$ rate constants from information available from patch-clamp data depended upon the assignment of nonzero rate constants to their correct values. We know of no case where such information would be available. Typically, pairs of rate constants are arbitrarily set to zero, i.e., some transitions are forbidden. If we analyzed data generated by models of the form of Fig. 1 *B* after assigning $q(1,3)$, $q(3,1)$, $q(2,4)$, and $q(4,2)$ to their zero values, the remaining eight rate constants are each specifically calculated, yielding the values used to generate the data. On the other hand, if $q(1,4)$, $q(4,1)$, $q(1,3)$, and $q(3,1)$ are fixed at zero values, it is not possible to calculate unique values for the remaining eight rate constants. Instead, there are multiple solutions for these parameters. Thus, not all sets of G rate constant assignments specify H rate constants. Eq. 46 apparently reveals the degrees of freedom among the W constructs only when intraconductance rate constants are given assigned values. Fixing a different set of G rate constants may or may not yield a specific solution for the calculated rate constants.

Fredkin and Rice (1986) considered the rank of the dwell time density amplitude matrices $\mathbf{A}_{oc}$ and $\mathbf{A}_{co}$ with regard to the number of independent experimental parameters. We considered the possibility that the failure of some sets of G assignments to specify the remaining rate constants might be due to the fact that, for the data generated by model 1 *B*, the ranks of $\mathbf{A}_{oc}$ and $\mathbf{A}_{co}$ are reduced to one, instead of having the full rank of 2. This turns out not to be the case since data generated by the rate constants listed in Table I, column 3 also yielded amplitude matrices with ranks of 1, but assignment of $q(1,3)$, $q(3,1)$, $q(1,4)$, and $q(4,1)$ allowed the specific calculation of the remaining eight rate constants. Thus, for the method we use to calculate rate constants, the ranks of the dwell time density amplitude matrices do not determine the number of rate constants that can be calculated from the information of patch-clamp data. We are presently investigating this question further. In summary, we have shown that assignment of the G intraconductance rate constants allows the specification of the remaining H rate constants for any data, regardless of the rank of the amplitude matrices. Fixing any G rate constants specifies the H other rate constants for many, but not all, data.

We have also considered a three conductance model with four kinetic states where two of the conductances have single kinetic states, and one conductance has two kinetic states (Fig. 1 *C*). Eq. 46 predicts that we must assign 2 rate constants. We produced three point joint probability amplitudes and eigenvalues for such a model as described above. Making the two rate constant assignments for the transitions between the kinetic states of the conductance that had two kinetic states was sufficient to specify the other 10 rate constants.

A consequence of not being able to calculate all of the rate constants is that models can be found that differ by their assigned rate constant values, but produce the same data. For example, rate constant values for the models shown in Fig. 2 can be found which produce the identical **W** constructs and eigenvalues (Table II). Models that fit the same **W** constructs and eigenvalues also produce identical two-dimensional dwell time density parameters, demonstrating that three-point joint probability parameters have all of the information that is available in patch clamp data. Note that while the models shown in Fig. 2, *A*–*C* are in thermodynamic equilibrium and have only one gateway between the closed and open states, the model in Fig. 2 *D* is not in thermodynamic equilibrium and has a complicated interaction among all four kinetic states. Yet all of these models produce the same patch-clamp data. Thus, in general, it is not possible to determine from patch-clamp data if the mechanism underlying channel gating is in thermodynamic equilibrium nor is it possible to determine the pathways of channel gating (see Discussion).

PART III: KINETIC ANALYSIS OF NONSTATIONARY PATCH-CLAMP DATA

A. Dwell Time Analyses

The Maximum Number of Independent Parameters in Nonstationary Data as Determined by Dwell Time Probabilities. Consider an experiment examining the kinetics of gating of a voltage-regulated channel where one records stationary channel behavior at a conditioning voltage, $V1$, and then the nonstationary gating in response to a step to a test voltage $V2$, at time τ_1. The event of interest here is the probability that the channel is in conductance I from time $t = 0$ to time τ_1, stays in conductance I for a time τ_2, and then changes to some other conductance. We define this probability as $P_{V1,V2}(\tau_1,\tau_2)$, which is given by

$$P_{V1,V2}(\tau_1,\tau_2) = \mathbf{u}_I'\mathbf{S}_{I,V1}\mathbf{G}_{I,V1}(\tau_1)\mathbf{Y}_{I,V1,V2}\mathbf{G}_{I,V2}(\tau_2)\mathbf{T}_{I,V2}\mathbf{u}_I = \mathbf{u}_I'\mathbf{G}_{I,V1}(\tau_1)\mathbf{M}_{I,V1,V2}\mathbf{G}_{I,V2}(\tau_2)\mathbf{u}_I, \quad (70)$$

where

$$\mathbf{Y}_{I,V1,V2} = \mathbf{R}_{I,V1}\mathbf{C}_{I,V2} \quad (71)$$

$$\mathbf{M}_{I,V1,V2} = \mathbf{S}_{I,V1}\mathbf{Y}_{I,V1,V2}\mathbf{T}_{I,V2} \quad (72)$$

and the constructs **S**, **T**, and **G** are as defined earlier, with additional subscript descriptions as to their voltage. Dwell times before and after this event include only information obtainable from stationary data.

For each I, there are N_I by N_I constructs M, which are the amplitudes to the dwell time density of nonstationary data. The information in nonstationary data that is not in stationary data is in these amplitudes.

Note that the reverse protocol from $V2$ to $V1$ produces the construct

$$\mathbf{Y}_{I,V2,V1} = \mathbf{R}_{I,V2}\mathbf{C}_{I,V1} = \mathbf{Y}_{I,V1,V2}^{-1} \quad (73)$$

and yields no additional information.

There is at least the following interdependence among these amplitudes:

$$\begin{aligned}
&-\mathbf{M}_{I,V1,V2}\mathbf{\Lambda}_{I,V2}^{-1}\mathbf{u}_I = -\mathbf{S}_{I,V1}\mathbf{Y}_{I,V1,V2}\mathbf{\Lambda}_{I,V2}^{-1}\mathbf{T}_{I,V2}\mathbf{u}_I \\
&= \mathbf{S}_{I,V1}\mathbf{R}_{I,V1}\mathbf{C}_{I,V2}\mathbf{\Lambda}_{I,V2}^{-1}\mathbf{\Lambda}_{I,V2}\mathbf{R}_{I,V2}\mathbf{u}_I \\
&= \mathbf{S}_{I,V1}\mathbf{R}_{I,V1}\mathbf{u}_I \\
&= -\mathbf{\Lambda}_{I,V1}^{-1}\mathbf{S}_{I,V1}(-\mathbf{\Lambda}_{I,V1}\mathbf{R}_{I,V1}\mathbf{u}_I) \\
&= -(-\mathbf{\Lambda}_{I,V1}^{-1}\mathbf{S}_{I,V1}\mathbf{T}_{I,V1}\mathbf{u}_I) \\
&= -\mathbf{\Lambda}_{I,V1}^{-1}\mathbf{V}_{I,V1}\mathbf{u}_I
\end{aligned} \quad (74)$$

by Eqs. 75, 71, 36, 23, and 39. The right-hand side of Eq. 74 consists of a product of terms known from stationary analysis. The above equation places N_I constraints on the amplitudes M, for each I. That is, there is N_I less "new" information for each I.

Summing up the independent amplitudes, we have a maximum of

$$\sum_{I=1}^{x} N_I N_I - \sum_{I=1}^{x} N_I = \sum_{I=1}^{x} N_I(N_I - 1) = G \quad (75)$$

new pieces of information (compare with Eq. 46). Thus out of a system of $2n(n-1)$ rate constants [$n(n-1)$ for each voltage], we can obtain H pieces of information from stationary data at each voltage, plus the G additional pieces of information from nonstationary data, or a maximum of

$$H + H + G = H + n(n-1) \quad (76)$$

since $H + G = n(n-1)$. The significance of this will be considered after considering the analysis of nonstationary data via point joint probabilities.

B. Point Joint Probability Analyses

Analysis of Nonstationary Data by the Point Joint Probability Method. We now consider analysis of nonstationary data via point joint probabilities. Again, the membrane is held at $V1$ until time τ_1, when it is jumped to $V2$. We consider the three-point joint probability formed from the probability that a channel is in conductance I at some moment t while the membrane is held at $V1$, that it is

in conductance J a time τ_1 later just before transition to potential $V2$, and then is in conductance K a time τ_2 after the potential change. The exponential constants to this probability are the eigenvalues Λ_{V1} for τ_1 and Λ_{V2} for τ_2, where the subscripts refer to voltage. An amplitude for such a three-point analysis is (by modification of Eq. 55):

$$a_3(m1,m2) = w_{I1,V1}(n,m1)w_{I2,V1,V2}(m1,m2)w_{I3,V2}(m2,n), \quad (77)$$

where the subscripts refer to the voltage with which the construct is associated and the construct $w_{I,V1,V2}$ defined as

$$w_{I,V1,V2}(m1, m2) = \sum_{i \in B(I)} r_{V1}(m1, i)c_{V2}(i, m2) \quad (78a)$$

or in matrix form,

$$\mathbf{W}_{I,V1,V2} = \mathbf{R}_{V1}\mathbf{\Gamma}_I\mathbf{C}_{V2}. \quad (78b)$$

Thus, the $\mathbf{W}_{I,V1,V2}$ are experimentally obtained from the amplitudes of a three-point joint probability with the middle point at the voltage transition. The $\mathbf{W}_{I,V1,V2}$ constructs contain not just information about the voltage transition, but also contain all of the information that stationary data has for $V1$ and $V2$, as shall be shown.

We first define the matrix $\mathbf{Z}_{V1,V2}$ as

$$\mathbf{Z}_{V1,V2} = \sum_{I=1}^{x} \mathbf{W}_{I,V1,V2} = \mathbf{R}_{V1}\left(\sum_{I=1}^{x} \mathbf{\Gamma}_I\right)\mathbf{C}_{V2} = \mathbf{R}_{V1}\mathbf{C}_{V2} \quad (79)$$

by Eq. 64c. Therefore,

$$\mathbf{Z}_{V2,V1} = \mathbf{R}_{V2}\mathbf{C}_{V1} = \mathbf{Z}_{V1,V2}^{-1}. \quad (80)$$

We now find the $\mathbf{W}$ stationary constructs for each voltage:

$$\mathbf{Z}_{V2,V1}\mathbf{W}_{I,V1,V2} = \mathbf{W}_{I,V2} \quad (81)$$

$$\mathbf{W}_{I,V1,V2}\mathbf{Z}_{V2,V1} = \mathbf{W}_{I,V1}, \quad (82)$$

which are derived from the definitions of the constructs (Eqs. 54, 76, and 79) and Eqs. 63 and 64. Since the $\mathbf{W}_{I,V1}$ and $\mathbf{W}_{I,V2}$ constructs contain all of the information in stationary patch clamp data (as far as amplitudes are concerned), we have shown that the $\mathbf{W}_{I,V1,V2}$ constructs have all stationary data information to each voltage.

In addition, we can obtain the $\mathbf{W}_{I,V2,V1}$ constructs of the reverse nonstationary protocol, from $V2$ to $V1$:

$$\mathbf{Z}_{V2,V1}\mathbf{W}_{I,V1,V2}\mathbf{Z}_{V2,V1} = \mathbf{W}_{I,V2,V1}, \quad (83)$$

showing that the protocol from $V2$ to $V1$ does not yield any additional information. Thus, analysis of three-point joint probability function from stationary and nonstationary data obtained before and just after a voltage jump obtains all of the kinetic information available from patch-clamp data.

We can rearrange Eq. 79 to yield the following equation:

$$\mathbf{C}_{V1}\mathbf{Z}_{V1,V2} = \mathbf{C}_{V2}. \quad (84)$$

Thus if the eigenvector elements of $V1$ are known from rate constants (known or calculated) at $V1$, then the eigenvector elements of $V2$ can be calculated from nonstationary data between $V1$ and $V2$.

Eq. 84 and the results of the previous section may be summarized. First, for a set of $n(n-1)$ known rate constants describing channel behavior at $V1$, one and only one solution for the $n(n-1)$ rate constants at $V2$ can be obtained from nonstationary data between the two voltages. Second, if G rate constant assignments at $V1$ are made that result in the specification of the other H rate constants at $V1$, then all $n(n-1)$ rate constants at $V2$ can be calculated from nonstationary patch-clamp data. Thus, a total of $H + n(n-1)$ rate constants are calculated with no remaining degrees of freedom, which is the maximum number of rate constants determinable from a complete stationary and nonstationary analysis between $V1$ and $V2$.

The three-point joint probability equations suggest an alternative approach to the analysis of nonstationary patch-clamp data. One can perform a complete analysis of stationary data obtained at $V1$ to determine the $\mathbf{W}_I$ constructs at $V1$. Then, an analysis of nonstationary data that considers only the probability of the channel being in a particular conductance at τ_1 before the voltage transition and at τ_2 after the voltage transition, without regard to the conductance at the voltage transition, is sufficient to obtain all of the kinetic information. The amplitudes from such an alternative analysis of nonstationary data are

$$a_3(m1,m2) = w_{I1,V1}(n,m1)z_{V1,V2}(m1,m2)w_{I3,V2}(m2,n). \quad (85)$$

From this the $\mathbf{Z}_{V1,V2}$ constructs can be obtained, and together with the $\mathbf{W}_{I,V1}$ constructs from stationary analysis at $V1$ (and eigenvalues at each voltage), the information would be complete: from $\mathbf{W}_{I,V1}$ and $\mathbf{\Lambda}_{V1}$ the H rate constants to $V1$ can be calculated, and from $\mathbf{Z}_{V1,V2}$ and $\mathbf{\Lambda}_{V2}$ the $n(n-1)$ rate constants for $V2$ can be calculated via Eqs. 84 and 2.

C. Examples of the Kinetic Analysis of Nonstationary Patch-Clamp Data

We generated three-point joint probability amplitudes from two sets of rate constants belonging to the general three-state, two conductance model (Fig. 1*D*). The two sets of rate constants correspond to rate constants at two voltages. In addition, we generated three point joint probability amplitudes in accordance with Eq. 77, for a time τ_1 before the voltage transition, at the transition to $V2$, and a time τ_2 after the transition. After G assignments were made at $V1$, in accordance with Eq. 46, we could calculate specific values for the remaining H rate constants at $V1$, plus all $n(n-1)$ rate constants at $V2$.

PART IV: PROCEDURES FOR THE ANALYSIS OF PATCH-CLAMP DATA

The two-dimensional dwell time density $P_{IJ}(\tau_1,\tau_2)$ can be obtained empirically by counting the number of times that

the channel had conductance $b(I)$ for a time τ_1 and subsequently had conductance $b(J)$ for a time τ_2. This would require $x(x-1)$ two-dimensional arrays $Ar_{IJ}(y,z)$, for all I and J except $J = I$. The indexes to the arrays refer to the bin number, and are related to the dwell times by $\tau_1 = hy$ and $\tau_2 = hz$, where h is the conversion factor in seconds per bin interval. The arrays are then least squares fitted, or maximum likelihood fitted, to obtain the amplitudes and exponential constants to the two-dimensional dwell time density.

For dwell time analysis of nonstationary data, data are collected counting the number of times that a channel had conductance $b(I)$ for a time τ_1 before the voltage transition, and continued to have conductance $b(I)$ for a time τ_2 after the voltage transition. The arrays $Ar_I(\tau_1,\tau_2)$ containing these frequencies are least squares fitted or maximum likelihood fitted to obtain the amplitudes and exponential constants to the two-dimensional dwell time density.

The empirical representation of the three-point joint probability $P_{SJJ}(\tau_1,\tau_2)$ is obtained by counting the number of times that a channel had conductance $b(S)$ at one moment, conductance $b(J)$ at some time τ_1 later, and conductance $b(J)$ at some time τ_2 thereafter. These frequencies are accumulated in the array $Ar_J(y,z)$, for $x-1$ of the J conductances. The y and z indexes are related to the τ's by $\tau_1 = hy$ and $\tau_2 = hz$, where h is the conversion factor in seconds per consecutive sampling point of the digitized patch-clamp data. The arrays $Ar_J(y,z)$ containing these frequencies are least squares fitted or maximum likelihood fitted to obtain the amplitudes and exponential constants to the three-point joint probability.

For point probability analysis of nonstationary data, the data are collected counting up the number of times that the channel had conductance $b(S)$ at a time τ_1 before the voltage transition, had conductance $b(J)$ at the voltage transition, and had conductance $b(J)$ at a time τ_2 after the voltage transition. Thus, the procedure is the same as for the analysis of stationary data, but the middle point is always at the voltage transition. For the alternative point joint probability analysis of nonstationary data described earlier, the conductance of the channel at the voltage transition is ignored.

By a numerical analysis procedure similar to that described in the section "Examples of the Kinetic Analysis of Stationary Patch-Clamp Data," the rate constants can be calculated from the amplitudes, exponential constants, and assignments of the particular analysis.

Two-dimensional data analyses are clearly more demanding than one-dimensional analyses. Their feasibility and implementation are currently being investigated (Bauer, R.J., manuscript in preparation).

DISCUSSION

We have determined the amount of kinetic information available from analysis of stationary and nonstationary patch-clamp data, the forms of analysis that extract that information, and the ability of that information to distinguish among various forms of n state kinetic schemes. This work has been general in the context of modeling channel gating as a Markov process. Thus, we have considered the case of a channel that shows x different conductance levels and n different kinetic states. A future paper will consider the further generalization of the analysis of data with more than one channel (Bauer, R.J., manuscript in preparation).

We have considered patch-clamp data in terms of traditional dwell time analysis and also in terms of point joint probability functions. The latter approach was found to be as powerful as dwell time analysis in determining the amount of information available in patch-clamp data. However, the proofs using point joint probability analysis are rather shorter than those based on dwell time considerations. In addition, point joint probability functions are better suited for the analysis of data with more than one channel (Bauer, R.J., manuscript in preparation).

The Amount of Kinetic Information Available from Patch-Clamp Data

A complete kinetic model for channel gating requires a number n for the number of kinetic states, plus $n(n-1)$ rate constants for the transitions between pairs of those states. Most generally, for voltage dependent channels there are $n(n-1)$ rate constants at each voltage. Because patch-clamp data directly measure only the transitions between states with different conductance levels there is ambiguity in the data that prevents the determination of all of the rate constants from these data alone. We have shown that stationary patch-clamp data have information to determine no more than H rate constants where (Eq. 45):

$$H = 2\left(\sum_{I=1}^{x-1}\sum_{J=I+1}^{x} N_I N_J\right)$$

with N_I and N_J being the number of kinetic states associated with conductance levels I and J, respectively. Accordingly, in using stationary patch-clamp data to obtain these rate constants, $G = [n(n-1)] - H$ rate constants must be fixed on the basis of data obtained from some other source or, more commonly, assigned arbitrary values. Nonstationary patch-clamp data have information about the voltage dependencies of the rate constants that is not available from stationary data. We have shown that a complete analysis of nonstationary data will obtain no more than H rate constants describing channel gating at a reference voltage after G rate constants have been fixed, as described above, plus all $n(n-1)$ rate constants at all other test voltages.

These results are simplified in the special case of channels showing two conductance levels (open and closed) with only a single open state plus any number of closed states. In this case, the analysis necessary to obtain all of

the rate constants is simpler (see below) and

$$H = 2(n - 1)$$
$$G = (n - 2)(n - 1).$$

It is worth noting that for many channels H is small relative to $n(n - 1)$. For example, Blatz and Magleby (1986) described the gating of a chloride channel that showed five closed states plus two open states: $n(n - 1) = 42$. A complete kinetic analysis of stationary patch-clamp data from such a channel requires that 22 rate constants be fixed for the calculation of the remaining 20. Similarly, models of sodium channel gating often have four closed states plus a single open state. For such models there are 20 rate constants, 12 of which must be obtained from data from experiments other than patch-clamp (e.g., gating current measurements). From this point of view, patch-clamp data are not a particularly rich source of information about the kinetics of channel gating and, ultimately, most kinetic information must come from other forms of measurements.

Methods of Analysis

Standard one-dimensional dwell time histograms provide a complete kinetic analysis of patch-clamp data only for channels showing a single open state (cf. Lecar, 1986). For channels with more complicated behavior (e.g., sodium channels, Sigworth, 1981; calcium channels, Hess et al., 1984; acetylcholine receptor channels, Labarca et al., 1984; chloride channels, Blatz and Magleby, 1986) a more powerful analysis is needed. In general, all of the kinetic information is contained in two-dimensional dwell time histograms that plot the frequency of observation of sequential dwell times of durations τ_1 and τ_2 (Fredkin et al., 1985) or in the three-point joint probability function that a channel is in a given conductance at time t, and at time $t + \tau_1$, and again at time $t + \tau_1 + \tau_2$.

As noted earlier, for the special case of channels with a single open state, a one-dimensional analysis using either standard dwell time histograms or two-point joint probability functions will extract all of the kinetic information available from patch-clamp data. We mention that the two-point joint probability function is readily calculated via standard autocorrelation functions and that our preliminary experience with this method shows it to be a simple and rapid method for the kinetic analysis of patch-clamp data (Bauer and Kenyon, 1987).

Which Rate Constants Can Be Determined from Patch-Clamp Data?

Having established the number of rate constants that can be determined from patch-clamp data, there remains the question of which of the $n(n - 1)$ rate constants can be calculated. Our results indicate that some transitions are well described by patch-clamp data while others are not. For example, patch-clamp data obtained from a channel with a single open state plus multiple closed states provide an excellent characterization of the rate constants into and out of the open state while rate constants between closed states are less well characterized. Information about those transitions not well represented in patch-clamp data must be obtained elsewhere. Ideally, one would have an independent measurement of these transitions and use this information to fix the G parameters necessary for the calculation of the remaining H rate constants. In the absence of such information, the calculation of H rate constants requires the arbitrary assignment of G rate constants. Obviously, the resulting rate constants depend equally on the assignments and the patch-clamp data. Furthermore, a given set of G assignments may or may not resolve all of the ambiguity in the patch-clamp data. Assignments that do not will not be sufficient to provide for the calculation of H rate constants. This can be illustrated by considering the channel with a single open state described above. Clearly assignments that do no more than establish the mean open time do not provide any information about channel gating that is not in the data. We have described how, if the intraconductance rate constants are fixed and if there is a solution, there will be a countable finite set of rate constant solutions. In addition, if the intraconductance rate constants are set to zero, then a single set of H rate constants can be calculated from any set of patch-clamp data.

In considering the kinetic information available from patch-clamp data, it is useful to think in terms of a solution space to a particular set of patch-clamp data, rather than in terms of a unique and particular kinetic scheme with which the data are consistent, but whose validity cannot be proven. Thus, the kinetics of channel gating are described by $n(n - 1)$ rate constants which can be used to specify a point in an $n(n - 1)$ dimensional solution space. Patch-clamp data have G degrees of freedom with regard to the specification of points in this solution space and, in the absence of data with which to reduce those degrees of freedom, the analysis of patch-clamp data can specify nothing more constrained than a G dimensional surface in the $n(n - 1)$ dimensioned solution space. In terms of the mathematics of Markov processes, the points on the solution surface are indistinguishable and represent equivalent descriptions of the patch-clamp data. In this view, the ultimate goal of the kinetic analysis of patch-clamp data is the definition of the solution surface that contains the single point that is the kinetics of channel gating.

Beyond this, one may impose external constraints on the accepted solutions such as the requirement that the rate constants be non-negative and real. The number of possible models may be further constrained to those whose rate constants reveal a particular dependence on voltage, such as an exponential dependence. Such a dependence could serve as additional evidence needed to narrow possible models.

The imposition of an aesthetically pleasing, mechanistic interpretation to the solution surface imposes a restriction

on the solution surface, which limits the information that can be derived from patch-clamp data. Of course, while a particular mechanism cannot be proven, a particular mechanism is disproved if its solution surface does not intersect with the patch-clamp data solution surface. For example, suppose no model that is in thermodynamic equilibrium is a solution to the data. Then one can conclude that channel gating is not in thermodynamic equilibrium (see below). Similarly, if no model with a single pathway between open and closed states is a solution, then one can conclude that there are more than one such pathway (cf. Fredkin et al., 1985). However, we have demonstrated that if patch-clamp data can be described by particular models that are in thermodynamic equilibrium or show a single pathway between closed and open states, this does not prove that the channel has these characteristics, since models that do not have these properties may also fit the data.

As noted earlier, the common way of reducing the degrees of freedom is the arbitrary assignment of rate constants. Another common assignment is thermodynamic equilibrium for channel gating, i.e., $\phi(i)q(i,j) = \phi(j)q(j,i)$, for each state i and j. This condition is not a Markovian constraint, and is not included in our derivations; channel gating may be a Markov stochastic process, but may or may not be in thermodynamic equilibrium. Because the thermodynamic equilibrium conditions are counted among the assignments, the same philosophy applies as with all assignments to reduce the degrees of freedom. Namely, for a set of assignments, if the constraints imposed by the thermodynamic equilibrium conditions are consistent with the data, then the proposed model is valid as far as the single channel macroscopic (conductance) current data are concerned. However, this is a special selection of the assignments and, as we have shown, is not necessarily an inherent property of the data.

The three-point joint probability or two-dimensional dwell time density analyses contain the information found in the analysis of temporal asymmetry used by Cull-Candy and Usowicz (1987) to study patch-clamp data from channels with more than two conductances. In this analysis, one counts the number of times a channel went from conductance I to conductance J, and from J to I, over a long time period. These values are directly proportional to the first derivative of the three point joint probability $P_{IJ}(\tau_1,\tau_2)$ with respect to τ_1, evaluated at $\tau_1 = 0$ and $\tau_2 = 0$. Thus, if a statistical fit of the three-point joint probability is made ("Procedures for Analysis of Patch-Clamp Data" section), then a statistical mean and standard error of the average rates of transition from conductance I to J and from J to I can be obtained from it, by which it can be determined if, and to what degree, these two values are different.

The two-point joint probability is essentially a noise analysis procedure of single channel records. This is sufficient to obtain all of the information present in patch-clamp data if $x - 1$ of the conductances have one kinetic state each, and the xth conductance has one or more kinetic states. Otherwise, a three-point joint probability analysis is essential for obtaining all of the information in patch-clamp data. It should be noted that a three-point joint probability noise analysis of macroscopic current data is theoretically possible, and we are preparing a manuscript describing the relationship between rate constants and multichannel three-point joint probabilities. In this way, it may be possible to obtain as much kinetic information from macroscopic current records as it is from single channel records for all types of two conductance kinetic data.

The analysis of stationary data requires that the assignments result in calculated rate constants that are non-negative, real, and consistent with the data for the particular voltage. Because nonstationary data have additional information, the assignments at one of the voltages must result in non-negative and real rate constants at all voltages included among the data. Thus, nonstationary analysis imposes more rigid requirements for validity of a model.

Comparison of the Information Content of Stationary and Nonstationary Patch-Clamp Data

Nonstationary data contain more information than stationary data. Consider the information available from the analysis of stationary data at a single voltage $V1$ from a channel with two closed and one open state (Fig. 1 *D*). By Eq. 46, two rate constants must be assigned to calculate the other four. For example, $q(1,3) = q(3,1) = 0$ forms a C-C-O model, while $q(1,2) = q(2,1) = 0$ gives a C-O-C model. Patch-clamp data showing two conductance levels and three kinetic states will be equally described by either of these models and no analysis of stationary data can determine that one form is valid while the other is invalid. Stationary patch-clamp data cannot be used to distinguish between C-C-O and C-O-C models (cf. Sakmann and Trube, 1984; Keller et al., 1986).

However, a complete analysis of nonstationary data obtained among a set of voltages using the three-point joint probability procedure described earlier with the middle point at the voltage change, provides additional information about the change in rate constants with voltage for a particular model specified by G assignments made at one voltage. If the true values of $q(1,2)$, $q(2,1)$, $q(2,3)$, and $q(3,2)$ change with voltage while $q(1,3)$ and $q(3,1)$ are zero at all voltages considered, then a fortuitously correct assignment of $q(1,3) = 0$ and $q(3,1) = 0$ at any one voltage results in the calculation of the true rate constants at all voltages, i.e., $q(1,3)$ and $q(3,1)$ will be calculated to be zero at all voltages. Making a C-O-C assignment at any voltage [$q(2,1)$ and $q(1,2)$ are zero] will result in all six of the calculated rate constants changing with voltage. That is, a C-O-C relationship among the calculated rate constants will not be maintained at the test voltages. The converse is

also true, if the rate constants do change with voltage in a C-O-C manner, only a C-O-C model will fit the data at all voltages. Finally, if all six of the true rate constants vary with voltage, neither a C-C-O or C-O-C relationship will be maintained for the calculated rate constants at all of the voltages considered. Thus, nonstationary analysis can determine whether the kinetic system is C-O-C, C-C-O, or neither.

In summary, analysis of nonstationary data will not allow the calculation of more rate constants at some reference voltage, *V*ref, than can be obtained by analysis of stationary data. However, no additional assignments must be made for the analysis at other voltages. This means that for a kinetic system analyzed at v voltage steps, there are $vn(n-1)$ rate constants to be determined, but only H (at *V*ref) plus $(v-1)n(n-1)$ (at other voltages) independent parameters can be experimentally derived. This leaves G degrees of freedom among the whole set of voltages.

Stationary data has information to determine H independent parameters for each voltage, but it does not have all the information about the changes in rate constants between voltages that nonstationary data has. For stationary data, we obtain vH independent parameters or H per voltage, leaving vG degrees of freedom in the kinetic system. This analysis would require G assignments at each voltage, not just at one voltage.

Throughout this paper we have referred to assignments as the specification of a value to certain rate constants. In general, assignments can be any set of equations specifying relationships among rate constants, implicitly or explicitly, which are not provided under the assumptions of a Markov process. Furthermore, in the analysis of nonstationary data, the G assignments may be distributed among the voltages considered. This approach was used by Goldman and Hahin (1979) in their analysis of macroscopic sodium currents. They performed a complete nonstationary analysis of macroscopic currents between a conditioning potential ($V1$) and a test potential ($V2$), and fit the data to a three-state, two-conductance model. They made two assignments that incorporated the assumption of thermodynamic equilibrium at each voltage, i.e.,

$$q(1,2)q(2,3)q(3,1) = q(1,3)q(3,2)q(2,1)$$

(see Fig. 1 *D*), and calculated the remaining 10 rate constants. Thus, they specified the system with no remaining degrees of freedom. This is the only example in the literature that we know of where a nonstationary protocol was used to its maximum potential for obtaining all of the information available from transmembrane current measurements.

A Systematic Approach to the Kinetic Analysis of Patch-Clamp Data

The results described in this paper suggest a general and straightforward approach to the kinetic analysis of patch-clamp data. By inspection of the data one can determine the number of conductance levels, x, shown by the channel or channels. Dwell time histograms for each conductance level will show an exponential relaxation associated with the occupancy of each kinetic state with that conductance level giving a value for the number of kinetic states, n. Thus, the kinetic analysis of the data can yield all of the rate constants minus the G rate constants that must be obtained elsewhere. The number of unknowns, G, among the **W** constructs can be determined for a given set of data, as described earlier. After these G assignments have been made, the other rate constants can be determined by the specific analysis of dwell time histograms (Colquhoun and Sigworth, 1983; Fredkin et al., 1985) or point joint probability functions (Bauer and Kenyon, 1987). If the resulting rate constants are all non-negative and real then the model is valid. If the assignments result in a model that cannot be fit to the data, i.e., a solution cannot be found, then one or more of the assignments contradict the data and that particular form can be rejected.

Reporting Kinetic Data

From the preceding discussion, it is apparent that the values obtained for the rate constants depend on the assignments. Accordingly, we suggest that the results of patch-clamp experiments be reported in a form that is independent of these assignments. Specifically, the complete set of independent experimental parameters could be published for a set of data. For example, for stationary data, the **W** constructs (or the equivalent two-dimensional dwell time density amplitudes) may be submitted along with $n-1$ point joint probability time constants. For nonstationary data, in addition to the stationary information at a reference voltage, the $(n-1)(n-1)$ independent $\mathbf{Z}_{V1,V2}$ constructs (or the equivalent nonstationary dwell time density amplitudes) that relate the rate constant changes from the reference voltage to each of the other voltages could be reported, as well as the $(n-1)$ point joint probability time constants at each voltage.

Authors might also specify any assignments used to translate these experimental parameters into rate constants. In this way readers can use the experimental parameters and make their own assignments, or interpretations, yielding an alternate set of rate constants. In addition, other investigators may have measurements other than patch-clamp measurements that provide information about the correct assignments to make. They may then use the patch-clamp results performed by someone else and obtain rate constants that combine results from the different types of experiments.

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REFERENCES

Bauer, R. J., and J. L. Kenyon. 1987. The kinetic analysis of stationary patch-clamp data using autocorrelation techniques. *Biophys. J.* 51 (2, Pt. 2):246a. (Abstr.)

Blatz, A. L., and K. L. Magleby. 1986. Quantitative description of three modes of activity of fast chloride channels from rat skeletal muscle. *J. Physiol (Lond.).* 378:141–174.

Colquhoun, D., and A. G. Hawkes. 1977. Relaxation and fluctuations of 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 A. G. Hawkes. 1982. On the stochastic properties of bursts of single ion channel openings and of clusters of bursts. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 300:1–59.

Colquhoun, D., and A. G. Hawkes. 1987. A note on correlations in single ion channel records. *Proc. R. Soc. Lond. B. Biol. Sci.* 230:15–52.

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

Cull-Candy, S. G., and M. M. Usowicz. 1987. Multiple-conductance channels activated by excitatory amino acids in cerebellar neurons. *Nature (Lond.).* 325:525–528.

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 Neyman and Jack Kiefer. L. M. LeCam and R. A. Olshen, editors. Wadsworth Publishing Co., Belmont, CA. 269–289.

Fredkin, D. R., and J. A. Rice. 1986. On aggregated Markov processes. *J. Appl. Probab.* 23:208–214.

Goldman, L., and R. Hahin. 1979. Sodium conductance kinetics. Solution of the general, linear three-state model. *Cell Biophys.* 1:345–354.

Hamill, O. P., A. Marty, E. Neher, B. Sakmann, and F. J. Sigworth. 1981. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pfluegers Arch. Eur. J. Physiol.* 391:85–100.

Hess, P., J. B. Lansman, and R. W. Tsien. 1984. Different modes of Ca channel gating behaviour favoured by dihydropyridine Ca agonists and antagonists. *Nature (Lond.).* 311:538–544.

Horn, R., and K. Lange. 1983. Estimating kinetic constants from single channel data. *Biophys. J.* 43:207–223.

Horn, R., and C. A. Vandenberg. 1984. Statistical properties of sodium channels. *J. Gen. Physiol.* 84:505-534.

Isaacson, E., and H. B. Keller. 1966. Analysis of Numerical Methods. John Wiley & Sons, Inc., New York.

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.

Karlin, S., and H. M. Taylor. 1975. A First Course in Stochastic Processes. 2nd ed. Academic Press, Inc., New York. 117–166, 443–445.

Keller, B. U., R. P. Hartshorne, J. A. Talvenheimo, W. A. Catterall, and M. Montal. 1986. Sodium channels in planar lipid bilayers, channel gating kinetics of purified sodium channels modified by batrachotoxin. *J. Gen. Physiol.* 88:1–23.

Kerry, C. J., K. S. Kitts, R. L. Ramsey, M. S. P. Sansom, and P. N. Usherwood. 1986. Single channel kinetics of a glutamate receptor. *Biophys. J.* 50:367–374.

Labarca, P., J. Lindstrom, and M. Montal. 1984. The acetylcholine receptor channel from *Torpedo californica* has two open states. *J. Neurosci.* 4:502–507.

Lecar, H. 1986. Single-channel currents and postsynaptic drug actions. *In* Ionic Channels in Cells and Model Systems. R. Latorre, editor. Plenum Publishing Corp., New York. 17–36.

Sigworth, F. J. 1981. Covariance of nonstationary sodium current fluctuations at the node of Ranvier. *Biophys. J.* 34:111–133.

Sakmann, B., and G. Trube. 1984. Voltage-dependent inactivation of inward-rectifying single-channel currents in the guinea-pig heart cell membrane. *J. Physiol. (Lond).* 347:659–683.

Yeramian, E., A. Trautmann, and P. Claverie. 1986. Acetylcholine receptors are not functionally independent. *Biophys. J.* 50:253–263.