Yet another approach to the dwell-time omission problem of single-channel analysis

Serge C. Crouzy and Frederick J. Sigworth
Department of Cellular and Molecular Physiology, Yale University School of Medicine, New Haven, Connecticut 06510 USA

ABSTRACT Distortion of the open-time or closed-time distributions of single channel currents, due to limited time resolution of the recording system, has been addressed by many authors. The calculation of the modified distributions generally involves the numerical inversion of a Laplace transform and is difficult to apply in fitting multistate kinetic schemes to data. Our approach is to introduce "virtual states" into the kinetic scheme, as suggested by Blatz and Magleby (1986. *Biophys. J.* 49:967–980) to account for missed events. To simplify the assignment of rate constants in multistate schemes we make use of Kienker's (1989. *Proc. R. Soc. Lond.* 236:296–309) theory to first transform schemes to uncoupled form. Our approach provides a good approximation to the exact solution, while allowing the observable dwell-time distributions, and also the second-order probability density functions, to be computed by standard matrix techniques.

INTRODUCTION

Recordings of currents in single ionic channels are commonly analyzed in terms of the dwell times in closed and and open states. It has been pointed out by numerous authors that the limited time resolution of the recordings results in the distortion of the open- and closed-time distributions (Rickard, 1977; Sachs et al., 1982; Neher, 1983; Colquhoun and Sigworth, 1983; Magleby and Palotta, 1983). An example of the problem is illustrated in Fig. 1. A short open interval (epoch 3) and a short closed interval (epoch 6) are not detected, so that the apparent channel activity (part *B* of Fig. 1) shows extended closed and open intervals. In this figure, the usual model of the detection process is invoked, namely that a closed or open interval (an "e-dwell", cf. Colquhoun and Sigworth, 1983) is detected only if it begins with a dwell in that state longer than a particular dead time δ of the recording systems.

As was first shown by Roux and Sauvé (1985) and generalized by Ball and Sansom (1988) and Milne et al. (1988) it is possible to calculate the distributions of e-dwells, given a fixed dead time, from the activity of channels obeying any Markov gating scheme. The exact solution is difficult to apply in practice because it involves the numerical inversion of a Laplace transform. For this reason, several approaches have been taken to obtain approximate solutions. Roux and Sauvé (1985) presented a "first-order" solution that can be computed by standard matrix techniques but which ignores the contribution of the durations of missed events to the total duration of an observed dwell time. Milne et al. (1988) use a series expansion of the Laplace transform to obtain approximate solutions for simple kinetic models; they and Ball and Sansom (1988) also consider the use of moments of the dwell-time distributions, which are more readily computed. Blatz and Magleby (1986) take an alternative approach to the problem in which the values of certain rate constants in the kinetic scheme are modified to reflect the effect of missed events. Their technique yields the correct mean dwell times for simple kinetic schemes, but the application to multistate, coupled schemes requires the assumption that multiple state transitions do not occur during missed dwell times.

In this paper we present yet another approximate solution to this problem. We make the usual assumption that the limited time resolution of the single-channel recording is characterized by a dead time δ, such that all dwell times shorter than δ are not observed, but all longer dwell times are observed. Our approach is to introduce "virtual states" into the kinetic scheme, as suggested by Blatz and Magleby (1986); the transitions into and out of the virtual states correspond to transitions that are undetected experimentally. To avoid the difficulty of assigning virtual states in the case of complicated, coupled schemes we use the recent results of Kienker (1989) to allow any scheme to be transformed for the simple introduction of virtual states. The approximation in our solution is that we treat the missed dwell times as if they were dwells in single kinetic states and therefore have exponential probability distributions (the true distributions are exponentials that are truncated at the time δ). This approximation allows the observable dwell-time distributions to be computed by standard matrix tech-

0006-3495/90/09/731/13 $2.00

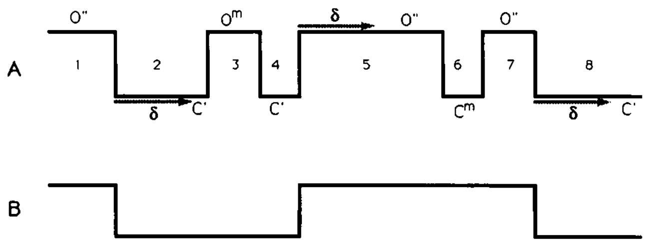


FIGURE 1 Idealized single-channel traces. (*A*) Channel activity including a brief opening (epoch 3) and a brief closure (epoch 6). (*B*) Apparent activity after imposing a dead time δ. Note that even though the closed time represented by epoch 4 in *A* is much shorter than δ, the e-opening in *B* does not begin until epoch 5, where a continuous opening longer than δ occurs. States of Schemes I′ and I″ corresponding to the channel activity are labeled in *A*.

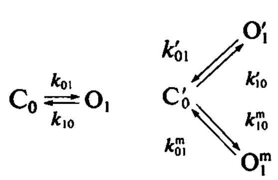
niques, while introducing errors that are expected to be negligible in practice.

THEORY

A two-state scheme

Let us first consider the simple case of a channel with two states, closed and open (Scheme I). In the analysis of the channel's behavior, missed dwells in the closed state cause the apparent open time to be extended, whereas missed open dwell times extend the apparent closed time. For now we consider only the problem of computing the e-closed time distribution. To do this we introduce the "virtual scheme" I' in which the missed openings are represented as sojourns in the state O_1^m whereas openings that are detected are represented by the state O_1'. It is possible for a channel in its closed state C_0' to make a transition into either O_1' or O_1^m, but transitions between O_1' and O_1^m do not occur. Using this scheme, apparent closed times are taken to be the times between leaving the "observable" open state O_1' and returning to it.

Scheme I Scheme I′



Let us call P_1^m the probability that an open dwell time is less than the dead time δ and is therefore missed:

$$P_1^m = \int_0^\delta k_{10} \exp(-k_{10}t)\, dt = 1 - \exp(-k_{10}\delta). \quad (1)$$

The mean duration of these missed open dwells lies between 0 and $\delta/2$ and is given by

$$\langle D_1^m \rangle = \frac{\int_0^\delta k_{10} t \exp(-k_{10}t)\, dt}{P_1^m} = \frac{1}{k_{10}} - \frac{\delta \exp(-k_{10}\delta)}{P_1^m}. \quad (2)$$

Using these quantities we can assign the rate constants in the virtual scheme as follows. First, the dwell time in the closed state is unchanged, so that the two rates leading from the closed state C_0' should sum to the original opening rate k_{01}. The relative probabilities of entering O_1^m and O_1' from the closed state are P_1^m and $1 - P_1^m$, respectively. From these considerations we obtain

$$k'_{01} = k_{01}(1 - P_1^m)$$

$$k_{01}^m = k_{01}P_1^m.$$

To make the mean time in the virtual state equal to the mean duration of missed events we assign

$$k_{10}^m = \frac{1}{\langle D_1^m \rangle}. \quad (3)$$

For our present purpose of determining the apparent closed times, the rate k'_{10} is arbitrary, as will be proved later.

Thus all of the rates in the scheme are determined, and the e-closed-time distribution can now be obtained through solving for the time spent in the aggregate of states $\{C_0', O_1^m\}$. An example of an e-closure is shown in Fig. 1. It corresponds to a dwell in state C_0' (epoch 2) followed by dwells in O_1^m (epoch 3) and C_0' (epoch 4) before exiting to an observed opening. It should be emphasized that, by definition, an e-closed interval must begin with a dwell of length δ in the closed state, and therefore cannot be shorter than δ. The distribution of e-closed times is therefore computed from the time of leaving the aggregate $\{C_0', O_1^m\}$ given that the system is in state C_0' at time δ.

The distribution of e-open times can be computed analogously by constructing a three-state virtual scheme but now with an extra state C_0^m representing the missed closed events. We give an example of this procedure in a later section.

Coupled and uncoupled schemes

Virtual schemes can be readily constructed for some kinetic schemes having multiple open and closed states. For example, in scheme U of Fig. 2, the effect of missed openings can be modeled by adding virtual states O_2^m and O_3^m in parallel to the existing open states. However, in schemes like scheme G of Fig. 3, it is difficult to add such virtual open states and specify the rate constants. The difficulty arises from the direct connection between the

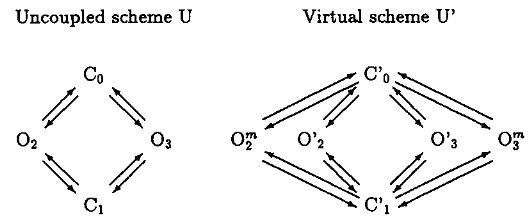


FIGURE 2 Construction of a virtual scheme U′ from an uncoupled scheme U to take into account missed openings. Dwells in the open states O_2 and O_3 that are shorter than the deadtime are represented by virtual closed states O_2^m and O_3^m, respectively. The states O_2' and O_3' in the virtual scheme account for detected open times.

open states, which implies that a missed opening can consist of dwells in both open states. In many such cases a reasonable approximation is to assume that missed dwell times consist of sojourns in a single state (Blatz and Magleby, 1986), and to compute effective rate constants on that basis. However, this approximation is not always valid, and it could be tedious to verify in every case.

Using Kienker's (1989) terminology, scheme G of Fig. 3 is called a "coupled" scheme, and U is an "uncoupled" scheme; an uncoupled scheme has no direct transitions between pairs of open states or between pairs of closed states. From any uncoupled scheme we can construct a virtual scheme having extra states representing missed dwell times. In some applications a "partially uncoupled" scheme will suffice. For example, if we are interested in computing the closed-time distribution in the presence of missed openings, we require only that the scheme be uncoupled for openings (Scheme Uo of Fig. 4) so that we can add virtual open states in parallel to the existing ones; coupling of the closed states does not matter in this case.

Transformation to uncoupled schemes

Kienker (1989) has demonstrated that through a class of similarity transformations it is possible to transform a

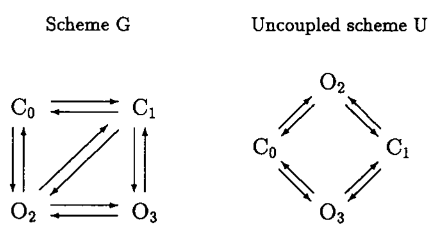


FIGURE 3 Example of a general, coupled scheme G and an uncoupled scheme U that could be made equivalent to it by the proper choice of rate constants. The schemes have the numbers of states $N_o = 2$, $N_c = 2$, and $N = 4$. The total number of identifiable rate constants is $H = 2N_oN_c = 8$. Although scheme G has 10 rates, U can be equivalent to it by proper choice of its $H = 8$ rate constants.

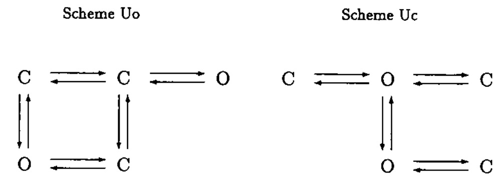


FIGURE 4 Example of a scheme uncoupled for openings (Uo) and a scheme uncoupled for closings (Uc).

given kinetic scheme into all other equivalent schemes which have indistinguishable kinetic behavior. Further, he showed that for a given scheme G there always exists a unique, equivalent uncoupled scheme. His results can be summarized as follows. Using the notation of Colquhoun and Hawkes (1981), we let **Q** be the matrix of transition rates of a given kinetic scheme which can be partitioned into the four submatrices $\mathbf{Q}_{oo}$, $\mathbf{Q}_{oc}$, $\mathbf{Q}_{co}$, and $\mathbf{Q}_{cc}$ which represent transitions among open states, between open and closed states, and transitions among closed states, respectively. Two kinetic schemes G and U are then equivalent if and only if their **Q** matrices are related by a similarity transformation

$$\mathbf{Q}_U = \mathbf{S} \cdot \mathbf{Q}_G \cdot \mathbf{S}^{-1} \tag{4}$$

in which **S** is an invertible block diagonal matrix in which each row sums to unity and has the form

$$\begin{pmatrix} \mathbf{S}_{oo} & \mathbf{O} \\ \mathbf{O} & \mathbf{S}_{cc} \end{pmatrix}.$$

Further, if the matrices $\mathbf{S}_{oo}$ and $\mathbf{S}_{cc}$ diagonalize the submatrices $\mathbf{Q}_{Goo}$ and $\mathbf{Q}_{Gcc}$ of $\mathbf{Q}_G$, then $\mathbf{Q}_{Uoo}$ and $\mathbf{Q}_{Ucc}$ will be diagonal. This is the condition for scheme U being uncoupled because then all rates among the open states and among the closed states are zero.

Assuming that $\mathbf{Q}_G$ is diagonalizable (which is true if the scheme satisfies detailed balance) then so are submatrices $\mathbf{Q}_{Goo}$ and $\mathbf{Q}_{Gcc}$.[1] The matrix **S** that yields the transformation to the uncoupled scheme is then fully and uniquely determined by letting $\mathbf{S}_{oo}$ be the matrix of eigenvectors of $\mathbf{Q}_{Goo}$ whose rows sum to unity, and $\mathbf{S}_{cc}$ be the matrix of eigenvectors of $\mathbf{Q}_{Gcc}$ whose rows sum to unity. Thus we can write, for the case of $\mathbf{S}_{oo}$,

$$\mathbf{Q}_{Uoo} = \mathbf{S}_{oo} \cdot \mathbf{Q}_{Goo} \cdot \mathbf{S}_{oo}^{-1}$$
$$\mathbf{S}_{oo} \cdot \mathbf{u} = \mathbf{u}$$

where **u** is a N_o by 1 column vector of ones.

The transformation to a partially uncoupled scheme

[1]If $\mathbf{Q}_{Gcc}$ (or $\mathbf{Q}_{Goo}$) is not diagonalizable because two or more eigenvalues are identical, then scheme G is lumpable and we should look for an equivalent scheme with fewer states.

can be performed similarly. For example, to transform G to a scheme uncoupled for openings, S_{oo} must be a matrix of eigenvectors of Q_{Goo} whose rows sum to unity, but S_{cc} could be the identity matrix.

Significance of uncoupled schemes

The states of an equivalent uncoupled scheme typically have no physical significance, but they have a mathematical significance. In a scheme uncoupled for openings, for example, the fact that the open states are connected only to closed states means that each dwell time in an open state is directly observable as an open interval. Because the dwell time in each state is exponentially distributed, each open state contributes an exponential component to the open-time distribution. Thus there is a one-to-one correspondence between each state in an uncoupled scheme and each exponential component of the dwell time distributions.

An uncoupled scheme obtained by Kienker's transformation is equivalent to the original scheme in that the distribution of dwell times in closed and open states, as well as the second-order probability density functions $f_{coc}(t_1, t_2)$ and $f_{oco}(t_1, t_2)$ are the same. Since Bauer et al. (1987) have shown that all other probability density functions for dwell times can be deduced from f_{coc} and f_{oco}, the uncoupled scheme behaves in a way indistinguishable from the original scheme. As Kienker points out, this is interesting in view of the fact that the schemes may have differing numbers of rate constants: an uncoupled scheme can have a maximum of only $2N_oN_c$ nonzero rate constants, which, however, is equal to the maximum number of identifiable rates (Bauer et al., 1987).

Negative transition rates in the uncoupled scheme sometimes result when strongly coupled schemes are transformed. Although they have no physical meaning, the negative rates are required to reproduce negative amplitudes of exponentials in the second-order probability density functions of some coupled schemes. The negative rates result in the correct formal solutions to the kinetic behavior; the sum of the rates leaving each state is always positive, so that the distribution of dwell times (first-order probability density function) in each state is a decaying exponential function.

Virtual states for a multistate scheme

We now consider the problem of computing the closed-time distribution for a multistate scheme when brief openings are missed. We start with a scheme that is uncoupled for openings such as scheme U of Fig. 2. Let the states be ordered such that $0, 1 \ldots N_c - 1$ are the N_c closed states and $N_c, N_c + 1 \ldots N_c + N_o - 1 = N - 1$ are the N_o open states. Let k_{ij} be the rate constant from state i to state j in the uncoupled kinetic scheme. The probability density function of dwell times in a given open state O_i is the single exponential

$$f_{o_i}(t) = \lambda_i \exp(-\lambda_i t),$$

where λ_i equals the sum of all rates leading away from state O_i, for $i = N_c$ to $N - 1$

$$\lambda_i = \sum_{j=0}^{N_c-1} k_{ij}. \tag{5}$$

Then the probability of missing an open dwell time in state O_i is simply

$$P_i^m = \int_0^\delta \lambda_i \exp(-\lambda_i t)\, dt = 1 - \exp(-\lambda_i \delta) \tag{6}$$

and the mean missed open time $\langle D_i^m \rangle$ is

$$\langle D_i^m \rangle = \frac{\int_0^\delta \lambda_i t \exp(-\lambda_i t)\, dt}{P_i^m} = \frac{1}{\lambda_i} - \frac{\delta \exp(-\lambda_i \delta)}{P_i^m} \tag{7}$$

for $i = N_c$ to $N - 1$. (Colquhoun and Sigworth, 1983; Neher, 1983; Blatz and Magleby, 1986).

As in the case of the two-state scheme considered earlier, we now create a virtual scheme U′ which has additional states to account for unobserved dwells in the actual open states. Our fundamental assumption, as before, is that we can approximate the distribution of missed dwell times by exponentially distributed dwells in the virtual states. Each original pathway in U from closed state C_j to open state O_i is replaced in the virtual scheme by two pathways (Fig. 2) representing the two possible outcomes of each closed → open transition:

(1) The dwell time in O_i is longer than δ. This is represented in U′ by a transition from closed state C_j' to detected open state O_i' ($j = 0$ to $N_c - 1$, $i = N_c$ to $N - 1$).

(2) The dwell time in O_i is shorter than δ. Then the transition to O_i will not be observed. In U′ this is represented by the transition from C_j' to the virtual state O_i^m ($j = 0$ to $N_c - 1$, $i = N_c$ to $N - 1$). The fact that U is uncoupled for openings implies that each virtual state O_i^m will also be uncoupled, i.e., it will only have pathways back to closed states.

Let us now derive the rate constants of the virtual scheme. The rates k_{ij}' and k_{ji}', $i = N_c$ to $N - 1$, $j = 0$ to $N_c - 1$ are for transitions between the detected open state O_i' and the closed state C_j'. The new set of rates k_{ij}^m and k_{ji}^m, $i = N_c$ to $N - 1$; $j = 0$ to $N_c - 1$ are for transitions between the missed open state O_i^m and the closed state C_j'. Then for $i = N_c$ to $N - 1$, $j = 0$ to $N_c - 1$, the following relations hold between the rates of the virtual scheme U′

and the scheme U:

$$k'_{ji} = k_{ji}(1 - P_i^{\mathrm{m}}) \qquad (8)$$

$$k_{ji}^{\mathrm{m}} = k_{ji}P_i^{\mathrm{m}} \qquad (9)$$

because when a transition occurs from closed state C_j to open state O_i it is detected with probability $1 - P_i^{\mathrm{m}}$. Meanwhile, to have the mean dwell time in state O_i^{m} be equal to the mean missed open time in O_i we have

$$\sum_{j=0}^{N_c-1} k_{ij}^{\mathrm{m}} = \lambda_i^{\mathrm{m}} \qquad (10)$$

with

$$\lambda_i^{\mathrm{m}} = \frac{1}{\langle D_i^{\mathrm{m}} \rangle}. \qquad (11)$$

The relative magnitudes of the rates k'_{ij} and k_{ij}^{m} leading from states O'_i and O_i^{m} can be obtained from the following consideration. The probability of a transition to state C_j given that the transition starts in O_i,

$$\pi_{ij} = \frac{k_{ij}}{\sum_{j=0}^{N_c-1} k_{ij}} \qquad (12)$$

will be equal to the corresponding probability π'_{ij} and π_{ij}^{m} for transitions starting from O'_i and O_i^{m}. Hence for $i = N_c$ to $N - 1$ and $j = 0$ to $N_c - 1$,

$$k'_{ij} = \alpha_i k_{ij} \qquad (13)$$

$$k_{ij}^{m} = \beta_i k_{ij}. \qquad (14)$$

From Eqs. 10 and 14 we find

$$\beta_i = \frac{\lambda_i^{m}}{\sum_{j=0}^{N_c-1} k_{ij}}. \qquad (15)$$

The value of α_i can be an arbitrary positive value because it determines the dwell time in the observed open state, a quantity that is irrelevant to us because we are constructing the virtual scheme for the determination of closed time. A proof of this assertion is given in the Appendix.

In the same way, we can create a virtual scheme U″ for estimating the e-open-time distribution. It has N_c additional states to account for missed closed times (Fig. 5). Starting with the uncoupled scheme, a matrix $\mathbf{Q}_{U''}$ is constructed, with N_c virtual open states; the matrix elements are computed as in Eqs. 8, 9, 13, and 14.

Although we have no proof for this, we think that detailed balance is preserved when we transform a general scheme G to an equivalent uncoupled scheme U. Moreover, from Eqs. 8, 9, 13, and 14 it is clear that microscopic reversibility is preserved when we change the equivalent scheme into the virtual schemes. Thus, if the initial scheme G satisfies detailed balance, we expect that the probability density functions of e-closed times and e-open times should be, after our approximation, a sum of decaying exponentials with positive amplitudes (Kijima and Kijima, 1987).

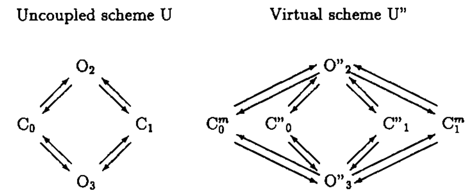


FIGURE 5 Construction of a virtual scheme U″ from an uncoupled scheme U to take into account missed closings. The significance of each state in U″ is readily understood by exchanging closed and open states in Fig. 2.

Computing the closed and open-time distributions

Having determined all of the rate constants of the two virtual schemes, we can use them to obtain the probability density functions $f'_c(t)$ and $f''_o(t)$ of the e-closed times and e-open times, respectively. First let us review the derivation of the pdfs in the case of no missed events. In the general case of a Markov process described by the transition matrix $\mathbf{Q}$ the closed dwell time probability density function is given by

$$f_c(t) = \pi_c e^{\mathbf{Q}_{cc}t}\mathbf{Q}_{co}\mathbf{u}_o, \qquad (16)$$

where π_c is the 1 by N_c row vector of equilibrium closed entrance state probabilities (Fredkin et al., 1985; Ball and Sansom, 1988). Its jth element is the steady-state probability that a closed interval begins in C_j, for $j = 0$ to $N_c - 1$. It is computed as the normalized sum over all open states i of the stationary probability to be in state i times the rate constant from i to j (Colquhoun and Hawkes, 1977):

$$\pi_c = \frac{\mathbf{P}_o^{\infty}\mathbf{Q}_{oc}}{\mathbf{P}_o^{\infty}\mathbf{Q}_{oc}\mathbf{u}_c},$$

where $\mathbf{P}_o^{\infty}$ is the vector of the stationary probabilities of the open states and $\mathbf{u}_o$ and $\mathbf{u}_c$ are column vectors of ones, one for each open or closed state, respectively.

The vector π_c can be alternatively written as the left eigenvector of matrix $\mathbf{Q}_{cc}^{-1} \cdot \mathbf{Q}_{co} \cdot \mathbf{Q}_{oo}^{-1} \cdot \mathbf{Q}_{oc}$ associated with the eigenvalue of unity, it satisfies

$$\pi_c \cdot \mathbf{u}_c = 1$$

and

$$\pi_c = \pi_c \cdot \mathbf{Q}_{cc}^{-1} \cdot \mathbf{Q}_{co} \cdot \mathbf{Q}_{oo}^{-1} \cdot \mathbf{Q}_{oc}. \quad (17)$$

Distributions from virtual schemes

Now let us compute the pdf of e-closed times using the virtual schemes. The virtual scheme U′ simulates missed openings through its N_o virtual states O_i^m, which are treated as closed states, yielding $N_c' = N_c + N_o$ closed states in all. From the submatrices of the transition matrix $\mathbf{Q}'$ of this scheme the pdf of e-closed times is obtained analogously to Eq. 16 as

$$f_c'(t) = \pi_c' e^{\mathbf{Q}_{cc}'(t-\delta)} \mathbf{Q}_{co}' \mathbf{u}_o \quad t \geq \delta, \quad (18)$$

and is zero for $t < \delta$.

Here π_c' is the 1 by N_c' row vector whose jth element is the steady-state probability that an observed closed interval begins in C_j', for $j = 0$ to $N_c' - 1$. Notice that the function is shifted in time by δ; as we discussed in the case of the two-state scheme, an e-closed interval always begins with a dwell of duration δ in a closed state (Fig. 1). Because the scheme U′ is uncoupled for closures, we know that if the e-closed interval begins in C_j', the system will remain in that state for the entire initial interval δ; thus π_c' is the appropriate probability function for the state of the system after the initial dwell time δ.

The closed entrance state vector π_c' is obtained analogously to π_c as described above, but makes use of the virtual scheme U′ and also U″, which describes the e-open intervals. The factors in Eq. 17 have the following significance: the first factor $\mathbf{Q}_{cc}^{-1} \cdot \mathbf{Q}_{co}$ refers to dwells in the closed states ending with a transition to an open state; in the case of missed events these dwells would be represented by the virtual scheme U′. The second factor, $\mathbf{Q}_{oo}^{-1} \cdot \mathbf{Q}_{oc}$, represents dwells in the open states ending with a transition to a closed state; in the case of missed events these dwells would be represented by the virtual scheme U″. Thus we can write the analogous eigenvector equation for π_c' as

$$\pi_c' = \pi_c' \cdot \mathbf{Q}_{cc}'^{-1} \cdot \mathbf{Q}_{co}' \cdot \mathbf{P}_{O' \to O''} \cdot \mathbf{Q}_{oo}''^{-1} \cdot \mathbf{Q}_{oc}'', \quad (19)$$

where $\mathbf{P}_{O' \to O''}$ is a projector from open states in virtual scheme U′ to open states in virtual scheme U″: $\mathbf{P}_{O' \to O''}$ is a matrix with N_o rows and N_o'' columns with its first N_o columns corresponding to the identity matrix and all its remaining columns zero. This projector means that we can only enter scheme U″, i.e., start an e-open time, in a true open state and not in a missed closed state. In the case of the four-state scheme given in Fig. 2, $\mathbf{P}_{O' \to O''}$ is:

$$\begin{pmatrix} 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 \end{pmatrix}.$$

The first N_c elements of vector π_c' are then computed as the left eigenvector associated with the unity eigenvalue of the transition matrix of closed entry process: $\mathbf{Q}_{cc}'^{-1} \cdot \mathbf{Q}_{co}' \cdot \mathbf{P}_{O' \to O''} \cdot \mathbf{Q}_{oo}''^{-1} \cdot \mathbf{Q}_{oc}''$ (Fredkin et al., 1985; Ball and Sansom, 1988), restricted to its N_c first rows. All following elements of π_c' are zero because an observed closed dwell cannot start in a missed open state. (The transition matrix has an eigenvalue of unity because all its columns sum to unity; all other eigenvalues are < 1 [Ball and Sansom, 1988]). Each element $[i, j]$ of the transition matrix is the probability that we observe a closed dwell time starting in observed closed state i, followed by some observed open dwell time ending in closed state j.

The corresponding equation for the probability density of the e-open times is

$$f_o''(t) = \pi_o'' e^{\mathbf{Q}_{oo}''(t-\delta)} \mathbf{Q}_{oc}'' \mathbf{u}_c \quad t \geq \delta, \quad (20)$$

where the row vector π_o'' of entrance-state probabilities is obtained from the equation

$$\pi_o'' = \pi_o'' \cdot \mathbf{Q}_{oo}''^{-1} \cdot \mathbf{Q}_{oc}'' \cdot \mathbf{P}_{C'' \to C'} \cdot \mathbf{Q}_{cc}'^{-1} \cdot \mathbf{Q}_{co}'.$$

Here $\mathbf{P}_{C'' \to C'}$ is a projector from closed states in virtual scheme U″ to closed states in virtual scheme U′ whose meaning is that we can only enter scheme U′ in a true closed state, not in a missed open state.

Fitting experimental data

Thus it is possible to obtain the pdfs of e-open times and e-closed times given the value of δ and the matrix of rate constants $\mathbf{Q}$ for an arbitrary Markov scheme. The calculation of these pdfs can be embedded in a fitting procedure to find the best set of rate constants, for example by maximizing the likelihood (Colquhoun and Sigworth, 1983). The fitting procedure would start with the scheme having an initial set of rate constants and would consist of repeating the following steps: (*a*) transform the original scheme to uncoupled form (if required); (*b*) build the virtual schemes; (*c*) solve for π_c' and π_o'', and then f_c' and f_o''; (*d*) compute the likelihood; (*e*) choose new values for rate constants in the original scheme.

Numerical implementation

We summarize here our numerical implementation of the calculations just described. Given a kinetic scheme G with N_o open states and N_c closed states, we first build the $\mathbf{Q}_G$ matrix and its four submatrices. If the scheme is coupled, we compute the transformation matrix $\mathbf{S}$ by finding the eigenvalues and eigenvectors of $\mathbf{Q}_{G_{oo}}$ and $\mathbf{Q}_{G_{cc}}$, for example using the QR algorithm (Press et al., 1986). By solving

Eq. 4, we now have the equivalent uncoupled scheme given by the matrix $\mathbf{Q}_U$.

To account for missed openings, we construct an enlarged matrix $\mathbf{Q}_{U'}$ by adding N_o virtual states, which are counted as closed states. The off-diagonal matrix elements are set equal to the new rate constants given in Eqs. 8, 9, 13, and 14, whereas the diagonal elements are chosen so that each row sums to zero (Colquhoun and Hawkes, 1977). A similar procedure is used to account for missed closings: we construct an enlarged matrix $\mathbf{Q}_{U''}$ by adding N_c virtual states, which represent missed closures and are counted as open states. Then we compute the product of matrices $\mathbf{Q}'^{-1}_{cc} \cdot \mathbf{Q}'_{co} \cdot \mathbf{P}_{O' \rightarrow O''} \cdot \mathbf{Q}''^{-1}_{oo} \cdot \mathbf{Q}''_{oc}$ and use a standard eigenvalue algorithm to derive π'_c as the eigenvector associated with the eigenvalue 1.0 of the transpose of this product restricted to its first N_c columns. Finally, from Eq. 18, the desired probability density function $f'_c(t)$ is determined for all $t \geq \delta$ (it is zero for $t < \delta$).

The matrix exponential in Eq. 18 is readily evaluated if the eigenvalues λ_i and the matrix of eigenvectors $\mathbf{M}$ of $\mathbf{Q}'_{cc}$ are first determined. Then f'_c can be written as a sum of N'_c exponentials

$$f''_c(t) = \sum_{i=1}^{N_c} a_i e^{\lambda_i (t-\delta)} \quad t \geq \delta, \quad \lambda_i \leq 0,$$

where

$$a_i = \sum_{l=1}^{N_c} \pi_{c'_l} m_{li} \sum_{j=1}^{N_o} \sum_{k=1}^{N_c} m_{ik}^{-1} q'_{co_{kj}}$$

and m_{li} and m_{ik}^{-1} are elements of M and its inverse, respectively.

The computations require only a moderate amount of computer time. The entire process for a 10-state scheme, starting with the 10×10 matrix $\mathbf{Q}$ and obtaining the a_i and λ_i, requires ~2 s in our implementation using the PowerMod Modula-2 compiler (Instrutech Corp., Elmont, NY) on a 68030-based computer.

Experimental data are usually fitted with exponential functions starting at $t = 0$, not at $t = \delta$. The normalized areas of the exponential components, extrapolated in this way back to zero time, are given by

$$A_i = \frac{a_i e^{-\lambda_i \delta}/\lambda_i}{\sum_{i=1}^{N'_c} [a_i e^{-\lambda_i \delta}/\lambda_i]}, \tag{21}$$

and each exponential $e^{\lambda_i t}$ will contribute to the mean observed closed dwell time according to

$$\mu_i = \int_\delta^\infty t f'_c(t)\, dt = \frac{a_i}{\lambda_i}\left[\frac{1}{\lambda_i} - \delta\right]. \tag{22}$$

RESULTS

Example of a two-state scheme

Closed time distribution

We first present an explicit solution for the two-state scheme I. The corresponding virtual scheme (I') has two closed and one open state and matrix $\mathbf{Q}_{U'}$ can be written, in the base (C'_0, O^m_1, O'_1),

$$\mathbf{Q}_{U'} = \begin{pmatrix} -(k'_{01} + k^m_{01}) & k^m_{01} & k'_{01} \\ k^m_{10} & -k^m_{10} & 0 \\ k'_{10} & 0 & -k'_{10} \end{pmatrix}$$

with explicit values for each rate given by Eqs. 1–3. In this very simple case, all closed dwell times will start in the only closed state C'_0 so that the vector of closed entrance probabilities will be $\pi'_c = (1, 0)$. Then, with the parameters

$$l_1 = 0.5\,(\lambda^m_1 - k_{01})$$

$$l_2 = 0.5\,(\lambda^m_1 + k_{01})$$

$$\alpha = \frac{\lambda^m_1 k_{01}(1 - P^m_1)}{l_2^2}$$

$$A = \frac{(1 - P^m_1)k_{01}}{2 l_2 \sqrt{1 - \alpha}}$$

the two eigenvalues of $\mathbf{Q}'_{cc}$ are given by

$$\lambda_1 = -l_2(1 - \sqrt{1 - \alpha})$$

$$\lambda_2 = -l_2(1 + \sqrt{1 - \alpha})$$

and the distribution of detected closed dwell times will be approximated by a sum of two exponentials:

$$f'_c(t) = A[l_1 + l_2\sqrt{1 - \alpha}]\exp[-\lambda_1(t - \delta)] + A[-l_1 + l_2\sqrt{1 - \alpha}]\exp[-\lambda_2(t - \delta)] \tag{23}$$

Open time distribution

The virtual scheme (I''), for computing the open-time distribution, has two open and one closed state. The corresponding matrix $\mathbf{Q}_{U''}$ can be written, in the base (C''_0, C^m_0, O''_1),

$$\mathbf{Q}_{U''} = \begin{pmatrix} -k''_{01} & 0 & k''_{01} \\ 0 & -k^m_{01} & k^m_{01} \\ k''_{10} & k^m_{10} & -(k''_{10} + k^m_{10}) \end{pmatrix}.$$

If P_0^m is the probability that a closed dwell time is less than the dead time,

$$P_0^m = 1 - \exp(-k_{01}\delta), \tag{24}$$

the mean duration of the missed closed dwells is given by

$$\langle D_0^m \rangle = \frac{1}{k_{01}} - \frac{\delta \exp(-k_{01}\delta)}{P_0^m}. \tag{25}$$

Then,

$$k''_{10} = k_{10}(1 - P_0^m)$$

$$k_{10}^m = k_{10}P_0^m,$$

$$k_{01}^m = \frac{1}{\langle D_0^m \rangle},$$

and the distribution of detected open dwell times: $f''_0(t)$ can be computed in the same way as the distribution of detected closed dwell times.

Numerical examples and comparisons with other solutions

In Fig. 6 we compare our approximate closed dwell time distribution (*dotted curve*) with Roux and Sauvé's (1985) exact solution (*solid curve*) for a symmetrical two-state

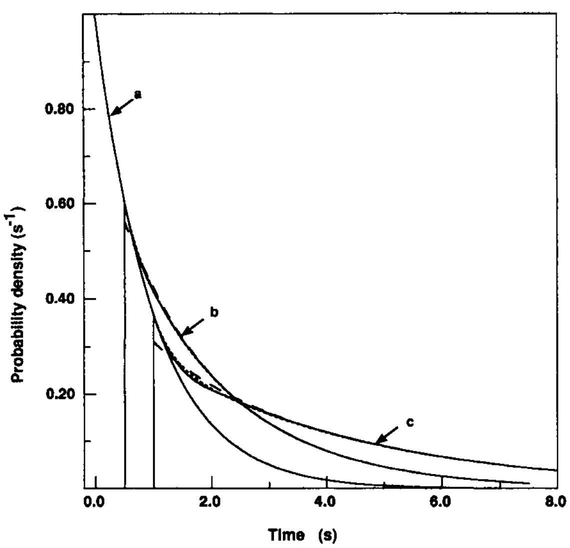


FIGURE 6 The solid curves show the exact solution for the distribution of e-closed times for a two state scheme with $k_{01} = k_{10} = 1\ s^{-1}$ and for three different values of the deadtime $\delta = 0$ (*a*), 0.5 (*b*), 1.0 (*c*) s. The corresponding approximate solutions obtained from Eq. 23 are plotted as dotted curves; the approximations presented by Yeo et al. (1988) are plotted as dashed curves.

scheme with $k_{01} = k_{10} = 1\ s^{-1}$ and $\delta = 0, 0.5$, and 1.0 s. Our two-exponential function is mostly obscured by the solid curve; it is larger than the true distribution for small t and slightly smaller for large t but the difference remains very small even for the extreme case of the deadtime equal to the mean dwell time. It should be noted that Roux and Sauvé did not take into account the fact that the probability density functions must be zero for times smaller than the dead time whereas we explicitly introduce this constraint by shifting the axis by δ. Therefore, we shifted the function as computed according to their theory by δ before displaying it. Also plotted in the figure as the dashed curve is the two-exponential approximation of Yeo et al. (1988), which also consists of two exponential terms, obtained from an expansion of the Laplace transform of the exact solution.

Let us now consider in detail the following asymmetrical two-state scheme:

$$C_0 \underset{k_{10} = 2\ s^{-1}}{\overset{k_{01} = 1\ s^{-1}}{\rightleftharpoons}} O_1 \qquad \text{II}$$

With a dead time $\delta = 0.5$ s, we build the virtual schemes II′ for computing the closed distribution and II″ for the open distribution,

$$O_1^m \underset{k_{01}^m = 0.6321\ s^{-1}}{\overset{k_{10}^m = 4.784\ s^{-1}}{\rightleftharpoons}} C'_0 \underset{k'_{10}:\ \text{"Free"}}{\overset{k'_{01} = 0.3679\ s^{-1}}{\rightleftharpoons}} O'_1 \qquad \text{II}'$$

$$C_0^m \underset{k_{10}^m = 0.7869\ s^{-1}}{\overset{k_{01}^m = 4.362\ s^{-1}}{\rightleftharpoons}} O''_1 \underset{k''_{01}:\ \text{"Free"}}{\overset{k''_{10} = 1.213\ s^{-1}}{\rightleftharpoons}} C''_0 \qquad \text{II}''$$

From these, we obtain

$$f'_c(t) = 0.3194 \exp[-0.3222(t - 0.5)] + 0.04851 \exp[-5.462(t - 0.5)]$$

$$f''_0(t) = 0.9326 \exp[-0.9839(t - 0.5)] + 0.2805 \exp[-5.378(t - 0.5)].$$

In Fig. 7, we show a histogram of 125,000 e-open times, obtained from a simulation of scheme II with $\delta = 0.5$ s. The prediction $f''_0(t)$ from the virtual scheme II″ is drawn as a dashed curve and compared with the best fit obtained with only one exponential (*solid curve*).

The two probability density functions $f'_c(t)$ and $f''_0(t)$ are also plotted in Fig. 8, *A* and *B*, respectively, as the dotted curves *a*. They follow very closely the exact solutions, (*solid curves*) obtained by FFT from Roux and Sauvé's theory (again shifted by δ).

Let us compare these results with some approximations that have been made for missed events for the simple C ⇌ O scheme. First, if the duration of a missed open interval is negligible compared with the mean closed dwell time,

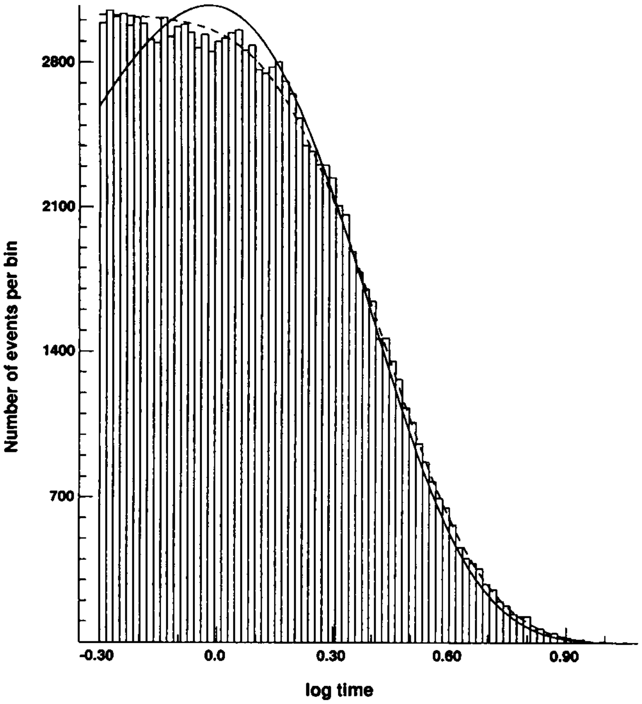


FIGURE 7 Dwell-time histogram built from 125,000 simulated observed open dwell times for the two-state scheme described in the text with $k_{01} = 1\ s^{-1}$ and $k_{10} = 2\ s^{-1}$ and with a deadtime $\delta = 0.5$ s. The best fit with only one exponential (*solid curve*), is compared with the two-exponential distribution (*dashed curve*) from Eq. 23.

i.e., $\lambda_1^m \gg k_{01}$, then Eq. 23 reduces to

$$f'_c(t) \simeq K \exp\left[-K(t-\delta)\right], \tag{26}$$

where

$$K = k_{01} \exp\left(-k_{10}\delta\right).$$

This is the standard result for the probability density function, of closed dwell times when open transitions shorter than δ are omitted (Sachs et al., 1982; Roux and Sauvé, 1985), and is plotted as curve b in Fig. 8 A. ($f''_0[t]$, following the same approximation, is shown as curve b in Fig. 8 b).

From Eq. 23, or directly from Eq. 22, we can easily derive the mean dwell time in the observed closed states (true closed plus missed open) as

$$DM_c = \int_\delta^\infty t f'_c(t)\, dt.$$

Then, assuming that the observed intervals are exponentially distributed with time constant τ_{obs} we deduce

$$\tau_{obs} = DM_c - \delta,$$

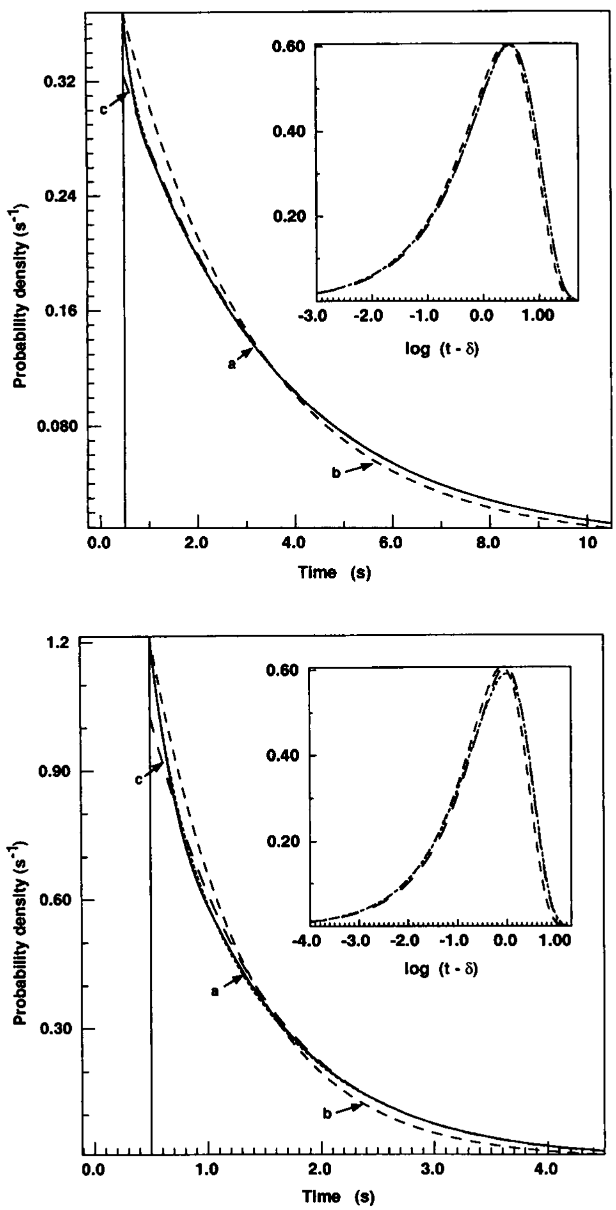


FIGURE 8 (A) Three approximations for the distribution of e-closed times shown together with Roux and Sauvé's exact solution shifted by δ (*solid curve*) in the case of the same two-state scheme as in Fig. 7. Curve a (dots nearly hidden by *solid curve*), our two-exponential distribution; Curve b (*short dashes*), the one-exponential approximation with $K = 0.3679\ s^{-1}$; Curve c (*long dashes*), single exponential with rate constant $K_{eff} = 0.3249\ s^{-1}$, computed according to Blatz and Magleby. The inset represents distributions a, b, and c plotted on a log-time axis (Sigworth and Sine, 1987; the abscissa is log $[t - \delta]$). (B) Comparison of the corresponding distributions of open dwell times. $K = 1.213\ s^{-1}$, $K_{eff} = 1.028\ s^{-1}$.

and an effective rate constant

$$K_{\text{eff}(01)} = 1/\tau_{\text{obs}}$$
$$= \frac{k_{01}k_{10}(1 - P_1^m)}{(k_{10} + k_{01}) - k_{01}(1 + \delta k_{10}) \exp(-k_{10}\delta)} \quad (27)$$

which is the approximation presented by Blatz and Magleby (1986) for the effective rate constant from closed to open. The resulting single exponential functions

$$f'_c(t) \simeq K_{\text{eff}(01)} e^{-K_{\text{eff}(01)}(t-\delta)}$$

$$f''_o(t) \simeq K_{\text{eff}(10)} e^{-K_{\text{eff}(10)}(t-\delta)}$$

are shown as curves c in Fig. 8, A and B, respectively.

In comparing the approximations shown in Fig. 8, we note that the condition $\lambda_1^m = 4.784\ \text{s}^{-1} \gg k_{01} = 1.0\ \text{s}^{-1}$ is not quite satisfied, which causes the approximation of Eq. 26 (curve b) to be a poor one. Blatz and Magleby's approximation (curve c) is much better, with a substantial deviation only for $t < \delta$, i.e., for dwell times that are normally not observable.

To check that our method gives relevant results for schemes with more than two states, we first considered Scheme III of the Ca activated K channel given by Blatz and Magleby (1986). As shown in Table 1 we obtained very similar values for the time constants and normalized areas (Eq. 21) of the slower exponential components. The extra exponentials introduced in the solution of the virtual scheme have very small areas and short time constants; this is to be expected in a case like this where the deadtime of 0.15 ms is small compared with the mean dwell times in closed or open states.

We also considered the four-binding-site model of the gating behavior of the locust muscle glutamate receptor, for which Ball and Sansom (1988) obtained numerical results for mean dwell times, transition-matrix eigenvalues and entrance-state probabilities. Although the initial kinetic scheme was strongly coupled and contained ten states (so that we had to build virtual schemes with fifteen states), we obtained identical values, up to the fourth significant figure, of each of these quantities as given in Tables 1–3 of their paper. We believe that our approximation preserves the correlation between open and closed times in multistate schemes; the agreement of the transition matrix and entrance-state probabilities is consistent with this. Further, as we will now show, it is possible to compute two-dimensional distributions from the virtual schemes.

Two-dimensional open-closed time distributions

The two-dimensional probability density functions $f_{c'o''}(t_1, t_2)$ gives the joint probability of an e-closed interval t_1 followed by an e-open interval t_2. The standard result for the probability density $f_{co}(t_1, t_2)$ when no events are missed is

$$f_{co}(t_1, t_2) = \pi_c e^{\mathbf{Q}_{cc}t_1} \mathbf{Q}_{co} e^{\mathbf{Q}_{oo}t_2} \mathbf{Q}_{oc} \mathbf{u}_c, \quad (28)$$

where π_c is the 1 by N_c row vector described previously and $\mathbf{u}_c$ is a column vector of ones.

The meaning of each term in this equation can be explained as follows: we start in a closed state with relative probability given by π_c; we spend time t_1 in the aggregate of closed states ($e^{\mathbf{Q}_{cc}t_1}$). Then there is a transition to one of the open states ($\mathbf{Q}_{co}$) and we spend time t_2 in the aggregate of open states ($e^{\mathbf{Q}_{oo}t_2}$) before returning to one of the closed states ($\mathbf{Q}_{oc}$).

The same arguments can now be used to derive $f_{c'o''}(t_1, t_2)$: we start in an observed closed state (in virtual scheme U′) with relative probability given by π'_c (Eq. 19); we spend a time t_1 larger than δ in the aggregate of observed closed states $[e^{Q'_{cc}(t_1-\delta)}]$. Then the system undergoes an observable transition to one of the open states; we

TABLE 1 Comparison between Blatz and Magleby's effective rate constants (effective rates) and our exponential distributions (virtual scheme) for a Ca activated K channel (Blatz and Magleby, 1986).

	τ_1	Area_1	τ_2	Area_2	τ_3	Area_3
Open Pdf	*ms*		*ms*		*ms*	
Effective rates	0.357	0.0997	5.49	0.900	—	—
Virtual scheme	0.3575	0.0968	5.495	0.9016	—	—
Extra terms	6.59 10^{-2}	8.31 10^{-4}	7.36 10^{-2}	7.51 10^{-4}	7.5 10^{-2}	9.0 10^{-6}
Closed Pdf						
Effective rates	0.230	0.801	1.96	0.130	47.0	0.0691
Virtual scheme	0.2314	0.7742	1.968	0.1275	46.95	0.0677
Extra terms	7.29 10^{-2}	3.06 10^{-2}	6.95 10^{-2}	−8.0 10^{-6}	—	—

The time constants and normalized areas are also given for the three extra open-time components and two extra closed-time components that arise in the virtual schemes.

take this into account with matrix $\mathbf{Q}'_{co}$ which describes transitions between closed states and an observable open state. This first open dwell, along with the rest of the transitions during the e-open time t_2 are now described with virtual scheme U″, i.e., $e^{\mathbf{Q}''_{oo}(t_2-\delta)}$. The channel finally undergoes an observable transition to one of the closed states, a transition that we take into account with matrix $\mathbf{Q}''_{oc}$. The resulting expression is

$$f_{c'o'}(t_1, t_2) = \pi'_c e^{\mathbf{Q}'_{cc}(t_1-\delta)}\mathbf{Q}'_{co} \cdot \mathbf{P}_{O'\rightarrow O''} \cdot e^{\mathbf{Q}''_{oo}(t_2-\delta)}\mathbf{Q}''_{oc}\mathbf{u}_c. \tag{29}$$

It should be noted that the transition matrix used to calculate π'_c, (Eq. 19) is proportional to the integral over all values of t_1 and t_2 from zero to infinity of $f_{c'o''}(t_1, t_2)$ (Fredkin et al., 1985).

We can explicitly write $f_{c'o''}(t_1, t_2)$ as a sum of exponentials of t_1 and t_2, for t_1 and $t_2 \geq \delta$:

$$f_{c'o''}(t_1, t_2) = \sum_{i=1}^{N'_c}\sum_{j=1}^{N''_o} a_{ij}e^{\lambda_i(t_1-\delta)}e^{\mu_j(t_2-\delta)},$$

where the λ_i's and μ_j's are the eigenvalues of matrices $\mathbf{Q}'_{cc}$ and $\mathbf{Q}''_{oo}$, respectively and

$$a_{ij} = \sum_{l=1}^{N_c}\pi_{c'_l}m_{li}\sum_{k=1}^{N'_c}\sum_{l=1}^{N_o} m^{-1}_{ik}q'_{co_{kl}}n_{lj}\sum_{p=1}^{N''_o}\sum_{q=1}^{N_c} n^{-1}_{jp}q''_{oc_{pq}}.$$

Here m_{ij} and n_{ij} are elements of M and N, the matrices of eigenvectors of $\mathbf{Q}'_{cc}$ and $\mathbf{Q}''_{oo}$, respectively; m^{-1}_{ij} and n^{-1}_{ij} are elements of M^{-1} and N^{-1}.

DISCUSSION

Our approximate solution to the missed-event problem gives very similar results to the exact solution in all cases that we have tried. The approximation, which involves treating missed dwell times as dwells in virtual states, yields the wrong probability distribution for the durations of the missed dwells. However, as we have shown, it is possible to assign the rate constants so that the mean dwell times in the virtual states equal the true mean durations of missed intervals. Because in most cases the missed dwell times make only a small contribution to the total observed dwell times, the difference in their distribution makes little difference in the overall, observed dwell-time distribution. Even in extreme cases where, for example, the mean closed time and the dead time δ are equal (Fig. 6*c*), the difference between the exact and approximate solutions is apparent only for closed times smaller than δ, which are normally not observable.

For comparison with experimental data, one normally wishes to compute both the closed-time distribution in the presence of missed openings, and the open-time distribution with missed closings. In our approach, the computations of the rate constants of the two distributions are done separately, with different virtual schemes being constructed to simulate the activity during extended closed and open intervals. It is possible, in the special case of a two-state process, to build a scheme with virtual states that accounts for both missed openings and missed closings. For example, the three-state virtual schemes II′ and II″ can be condensed into the following four-state scheme,

$$O^m_1 \underset{k^{m(')}_{01} = 0.6321\ s^{-1}}{\overset{k^{m(')}_{10} = 4.784\ s^{-1}}{\rightleftharpoons}} C'_0 \underset{k'_{10} = 1.213\ s^{-1}}{\overset{k'_{01} = 3.679\ s^{-1}}{\rightleftharpoons}} O'_1 \underset{k^{m('')}_{01} = 4.362\ s^{-1}}{\overset{k^{m('')}_{10} = 0.7869\ s^{-1}}{\rightleftharpoons}} C^m_0.$$

Here we have made use of the fact that rates k'_{10} and k''_{01} are both free parameters and therefore can be set to k''_{10} and k'_{01}, respectively. Considering $\{O^m_1, C'_0$ and $O'_1, C^m_0\}$ as new aggregates of closed and open states, respectively, we can solve for the eigenvalues and eigenvectors of this scheme and deduce both $f'_c(t)$ and $f''_o(t)$ in one step. It should be noted, however, that building such a condensed scheme is not possible for initial schemes with more than two states because the constraints on the rate constants of Eqs. 8 and 13 will not match in general.

Ambiguity in the determination of rates

It has been pointed out that the presence of missed events allows different sets of rate constants to yield the same observed mean open and closed times (Colquhoun and Sigworth, 1983). Yeo et al. (1988) have shown that higher-order approximations in the solution of the missed event problem can in principle remove the nonuniqueness of the solutions.

The origin of the nonuniqueness can be understood by considering the four-state virtual scheme above. Although in principle all eight rate constants for such a scheme can be determined from observed open and closed dwell times, the fact that the dwells in the virtual states are very short (by definition they represent missed events) means that in practice only one exponential component is seen in either the open or the closed time distribution. Even with the rate constants in the virtual scheme constrained by a known value of δ, there are two sets of values of k_{01} and k_{10} that yield the same time constants for the observable dwell time distributions. We expect that in practice this ambiguity is readily resolved by inspection of the original recordings; typically one solution represents a relatively low missed-event rate, whereas the other is for the case where the majority of events are missed.

Origin of the fast components

As can be seen in Fig. 6, the pdf of extended dwell times includes a rapidly decaying component when the probability of missed events becomes large. In our approximate solution this component consists of exponential components with time constants $\sim\delta$. Consider the case of e-closed intervals. By the criterion of detection illustrated in Fig. 1, this interval must begin with a time δ during which no missed openings occur. After this time, however, missed openings are allowed. In modeling this behavior we require that the channel occupies an observable closed state continuously for a time δ and only after this time the evolution of the system is modeled by a virtual scheme. The initial behavior of the virtual scheme is a rapid equilibration between the observable closed state and the states representing missed openings. The resulting rapidly decaying component consists of N_o exponentials with time constants generally smaller than $\delta/2$ and having small amplitudes.

Limitations of the theory

Although we have demonstrated a relatively good approximation to the exact solution of the missed-event problem, we wish to emphasize that the use of the methods that we and others have proposed are subject to three limitations in the way that the "missed-event problem" itself has been posed. First, the assumption is made that there is a clear and invariant criterion, namely the dead time, that determines whether a given dwell will be detected or not; and if it is detected, the proper duration will be measured. Many workers use the half-amplitude threshold detection technique, which in the absence of noise detects all events longer than about half of the filter risetime; for a Bessel or Gaussian filter the minimum event duration is $\delta \simeq 0.18/f_c$, where f_c is the -3 db frequency (Colquhoun and Sigworth, 1983). Due to the "damped filtering" this detector underestimates the duration of brief events that are detected, distorting the distribution of apparent dwell times (e.g., see Roux and Sauvé, Figs. 2 and 4). The approach in our laboratory has been to apply a correction to remove this distortion (see Colquhoun and Sigworth, 1983, Eq. 17). The presence of background noise in recordings means that the detection threshold is not sharp; simulations, however, show that the effect of noise on the observed distributions is not large (see Fig. 11–12 of Colquhoun and Sigworth, 1983).

The second assumption is that a transition is taken to be detectable only if the channel remains in the new state for at least the dead time. That is, in theory an e-opening is said to occur only if no brief closures occur during δ s. This is a simplified answer to the difficult problem of specifying the probability of detection in random series of transitions. As we discussed above, it is this feature of the specification of the missed-event problem that gives rise to the fast components of the predicted distributions; these components are most likely absent from actual data. The third issue is that, in practice, the probability of detection of a brief event is higher when it occurs as a cluster of closely-spaced events, due to summation in the filtering of the signal. This effect is not important in a recording when either the open or the closed dwell times are long, so that the brief events are well separated in time. In the situation where both open and closed times are brief and comparable to the dead time, the error from this effect might be substantial.

APPENDIX

We stated that $f_c(t)$ is unchanged when the factors α_i in Eq. 13 are set to any positive values. To prove this, let us first find a useful relation for π_c.

Let $\mathbf{P}^\infty$ be the row vector of stationary probabilities. It can be decomposed into $\mathbf{P}_o^\infty$ and $\mathbf{P}_c^\infty$, the stationary probabilities for being in the open and closed states, respectively. The relation

$$\mathbf{P}^\infty \cdot \mathbf{Q} = 0$$

from the general theory of Markov processes (Colquhoun and Hawkes, 1977) leads to the following:

$$\mathbf{P}_o^\infty \cdot \mathbf{Q}_{oo} + \mathbf{P}_c^\infty \cdot \mathbf{Q}_{co} = 0$$

$$\mathbf{P}_o^\infty \cdot \mathbf{Q}_{oc} + \mathbf{P}_c^\infty \cdot \mathbf{Q}_{cc} = 0$$

which can be combined into

$$\mathbf{P}_o^\infty = \mathbf{P}_o^\infty \cdot \mathbf{Q}_{oc} \cdot \mathbf{Q}_{cc}^{-1} \cdot \mathbf{Q}_{co} \cdot \mathbf{Q}_{oo}^{-1}$$

or

$$(\mathbf{P}_o^\infty \cdot \mathbf{Q}_{oc}) = (\mathbf{P}_o^\infty \cdot \mathbf{Q}_{oc}) \cdot \mathbf{Q}_{cc}^{-1} \cdot \mathbf{Q}_{co} \cdot \mathbf{Q}_{oo}^{-1} \cdot \mathbf{Q}_{oc}$$

and because

$$\pi_c = C \cdot (\mathbf{P}_o^\infty \cdot \mathbf{Q}_{oc}),$$

where C is a constant, we obtain the desired result (Kienker, 1989)

$$\pi_c = \pi_c \cdot \mathbf{Q}_{cc}^{-1} \cdot \mathbf{Q}_{co} \cdot \mathbf{Q}_{oo}^{-1} \cdot \mathbf{Q}_{oc}. \quad \text{(A1)}$$

Then, what happens to $f_c(t)$ when we change the rate constant from open state O_i to closed state C_j from k_{ij} to $k'_{ij} = \alpha_i k_{ij}$?

From Eq. 16, f_c depends on $\mathbf{Q}_{cc}$, $\mathbf{Q}_{co}$, and π_c. As $\mathbf{Q}_{cc}$ and $\mathbf{Q}_{co}$ are unchanged by the transformation, the exponential constants remain the same. Only π_c and therefore the amplitudes of the exponentials may change. Let us note by primes the matrices of the transformed scheme, and assume that the initial kinetic scheme is uncoupled for the openings, as will be the transformed scheme. $\mathbf{Q}_{oo}$ and $\mathbf{Q}'_{oo}$ are diagonal matrices whose element of row i equals the opposite of the sum of all elements on row i in $\mathbf{Q}_{oc}$ and $\mathbf{Q}'_{oc}$, respectively. Therefore, the diagonal element of row i in $\mathbf{Q}'_{oo}$ equals α_i times the diagonal element of row i in $\mathbf{Q}_{oo}$ and the diagonal element of row i in $\mathbf{Q}'^{-1}_{oo}$ equals $1/\alpha_i$ times the diagonal element of row i in $\mathbf{Q}_{oo}^{-1}$, for all i. Then,

$$\mathbf{Q}_{oo}^{-1} \cdot \mathbf{Q}_{oc} = \mathbf{Q}'^{-1}_{oo} \cdot \mathbf{Q}'_{oc} \quad \text{(A2)}$$

which implies by Eq. A1 that π_c and therefore $f_c(t)$ are unchanged by the transformation. Thus, α_i can be set to any convenient positive value, for all i equals N_c to $N - 1$.

This work was supported by grant NS-21501 from the National Institutes of Health.

Received for publication 11 January 1990 and in final form 21 May 1990.

REFERENCES

Ball, F., and M. Sansom. 1988. Aggregated Markov processes incorporating time interval omission. *Adv. Appl. Probab.* 20:546–572.

Bauer, R. J., B. F. Bowman, and J. L. Kenyon. 1987. Theory of the kinetic analysis of patch clamp data. *Biophys. J.* 52:961–978.

Blatz, A. L., and K. L. Magleby. 1986. Correcting single channel data for missed events. *Biophys. J.* 49:967–980.

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

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

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. 191–264.

Fredkin, D. R., M. Montal, and J. A. Rice. 1985. Identification of aggregated Markovian models: application to the nicotinic acetylcholine receptor. *In* Proc. Berkeley Conf. in Honor of J. Seyman and J. Kiefer. L. M. Le Cam and R. A. Olshen, editors. Wadsworth, Belmont, CA. 1:269–289.

Kienker, P. 1989. Equivalence of aggregated markov models of ion-channel gating. *Proc. R. Soc. Lond. Biol.* 236:269–309.

Kijima, S., and H. Kijima. 1987. Statistical analysis of channel current from a membrane patch, I. Some properties of ion channels or molecular systems in equilibrium. *J. Theor. Biol.* 128:423–434.

Magleby, K. L., and B. S. Palotta. 1983. Calcium dependence of open and shut interval distributions from calcium-activated potassium channels in cultured rat muscle. *J. Physiol. (Lond.).* 344:585–604.

Milne, R. K., G. F. Yeo, R. O. Edeson, and B. W. Madsen. 1988. Stochastic modelling of a single ion channel: an alternating renewal approach with application to limited time resolution. *Proc. R. Soc. Lond. Biol.* 233:247–292.

Neher, E. 1983. The charge carried by single-channel currents of rat cultured muscle cell in the presence of local anesthetics. *J. Physiol.* (*Lond.*). 339:663–678.

Press, W. H., B. P. Flannery, S. A. Teukolsky, and W. T. Vetterling. 1986. Numerical Recipes. The Art of Scientific Computing. Cambridge University Press, Cambridge. 335–380.

Rickard, J. T. 1977. The zero-crossing interval statistics of the smoothed random telegraph signal. *Info. Sci.* 13:253–268.

Roux, B., and R. Sauve. 1985. A general solution to the time interval omission problem applied to single channel analysis. *Biophys. J.* 48:149–158.

Sachs, F., J. Neil, and N. Barkakati. 1982. The automated analysis of data from single ionic channels. *Pfluegers Arch. Eur. J. Physiol.* 395:331–340.

Sigwoth, F. J., and S. M. Sine. 1987. Data transformations for improved display and fitting of single-channel dwell time histograms. *Biophys. J.* 52:1047–1054.

Yeo, G. F., R. K. Milne, R. O. Edeson, and B. W. Madsen. 1988. Statistical inference from single channel records: two-state Markov model with limited time resolution. *Proc. R. Soc. Lond. Biol.* 235:63–94.