A GENERAL SOLUTION TO THE TIME INTERVAL OMISSION PROBLEM APPLIED TO SINGLE CHANNEL ANALYSIS

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ABSTRACT To obtain the open or closed time interval distributions of patch clamp signals, several workers have used a half-amplitude minimum time interval criterion. Within this framework, no transition between states of different conductance levels is considered to have taken place if it leads to a time interval smaller than a certain critical value. This procedure modifies substantially the open or closed time interval distribution of the random signal to be analyzed, since time intervals well above the time resolution of the recording system may be interrupted by short gaps that may or may not satisfy the minimum time interval criterion. We present here a general theoretical framework by means of which the effect of time interval omission on time interval distributions can be taken into account. Based on the mathematical formalism provided by the Kolmogorov forward equation, special matrix operators are first defined. The general solution to the time omission problem in its integral form is then derived. In view of the poor computational feasibility of the resulting solution, a first-order approximation is also presented. This approximation consists essentially in neglecting the contribution of the undetected gaps to the total length of the resulting time interval. The exact and approximate solutions are then applied to two special kinetic schemes commonly found in single-channel studies, namely the O-C and C-O-C models. The applicability of the proposed formalism to the time interval distribution problem of a damped random signal is finally discussed.

INTRODUCTION

Since the introduction by Neher et al. (1978) of the extracellular patch clamp method (see also Hamill et al., 1981), electrophysiological studies of excitable and nonexcitable cells can now be carried out at the single channel level. There are essentially two basic parameters one can obtain from patch clamp experiments. For instance, an analysis of the amplitude of the current jumps can provide valuable information on the ionic permeability associated with a specific channel conducting state. However, this parameter alone does not enable one to relate the channel random openings and closings to a particular kinetic scheme. What is required then is to measure for a given random signal the distribution of open and closed time intervals, and to use the mathematical formalism provided by the continuous time Markov chain theory as a means to interpret the resulting distributions in terms of a specific kinetic model (see for example Conti and Wanke, 1975; Neher and Stevens, 1977; Colquhoun and Hawkes, 1977, 1981; De Felice, 1981).

There are, however, several problems related to the exact measurement of open or closed time intervals. Due to the finite time response of the recording system, it should be clear that very short intervals cannot be accurately measured. Some time intervals will simply remain undetected, whereas others will reflect more the time response of the recording system than the actual time interval distribution of the open or closed channel (see for instance the analysis of Colquhoun and Sigworth, 1983). It should also be clear that missing short time intervals will bias the overall estimate of the time interval distribution. For instance, if a channel opening is interrupted by a short gap, an undetected transition at this point will result in an apparent longer open time interval and thus in an overestimation of the channel open time interval probability density. This problem becomes especially important in cases where the signal-to-noise ratio is small, since low-pass filtering at low frequencies has then to be used to minimize the contribution of the background noise to the time interval distribution estimate.

To circumvent this particular problem, several workers have proposed a half-amplitude minimum time interval criterion (Sachs et al., 1982; Dionne and Leibowitz, 1982; Methfessel and Boheim, 1982; Moczydlowski and Latorre, 1983; Bechem et al., 1983, Sakmann and Trube, 1984). Within this framework, time intervals measured at half-amplitude and smaller than a predetermined value are

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simply neglected. Consequently, no transition is considered to have taken place if it leads to a time interval smaller than a certain critical value. It is assumed in most cases that this procedure will affect mostly the kinetic pathways corresponding to fast transitions (flickering) leaving undisturbed those pathways related to slower current fluctuations.

To our knowledge, there is in the literature no systematic analysis of the effect of time interval omission on the open or closed time interval distributions. Sachs et al. (1983) and Neher (1983) have discussed in detail the effect of time interval omission for the simple two-state open-closed kinetic scheme, but proposed no general treatment of this problem. One would like, however, to use for the purpose of analysis, independently of how complex the kinetic scheme related to a given channel may be, mathematical equations that take into account as accurately as possible the conditions under which the experimental data were obtained or selected for analysis. Therefore, we present here a general theoretical framework by means of which the effect of time interval omission on time interval distributions can be taken into account. The present approach should, in principle, lead to a more accurate estimation of the kinetic parameters associated with a particular kinetic scheme, since the computational problems coming from applying mathematical equations derived for an ideal signal to nonideal experimental data can be partly resolved.

We will first present the mathematical framework we intend to use to compute time interval distributions. Special matrix operators will be defined and known solutions to time-interval related problems will be derived. Second, the exact solution to the general time interval omission problem will be presented. In view of the complex mathematical form of the general solution, a first-order approximation procedure will also be introduced. This procedure will consist essentially in neglecting the contribution of the undetected gaps to the total length of the resulting time interval. Numerical calculations will then be used to validate the proposed approximation. Finally, the general formalism we obtained will be applied to two special kinetic schemes commonly found in single-channel studies.

GENERAL FORMULATION

It is now well established that the random openings and closings of a single ionic channel can satisfactorily be described in terms of a Markov process with discrete states in continuous time (Neher and Stevens, 1977; Colquhoun and Hawkes, 1977, 1981). The basic matrix equation for the conditional probability $P_{ij}(t)$ is thus given by the Kolmogorov forward equation, namely,

$$\frac{\mathrm{d}}{\mathrm{d}t} P(t) = P(t)\, Q, \tag{1}$$

where $P_{ij}(t)$ = probability (state j at time $[t + s]$/state i at time s), and where Q_{ij} represents the rate of transitions from the ith to jth state. It is now standard result that the formal solution of Eq. 1 can be expressed as[1]

$$\langle p(t)| = \langle p(0)|P(t) = \langle p(0)|e^{Qt}, \tag{2}$$

where $\langle \mathrm{p}(t)|$ is a row vector equal to $[\mathrm{p}_1(t), \mathrm{p}_2(t), \ldots \mathrm{p}_n(t)]$ in which $p_i(t)$ is the probability for the system to be in state i at t given that the initial probability vector was $[\mathrm{p}_1(0), \mathrm{p}_2(0) \ldots \mathrm{p}_n(0)]$, and

$$e^{Qt} = 1 + Qt + \frac{(Qt)^2}{2!}. \tag{3}$$

If we restrict ourselves to the special case of an ionic channel with only one possible nonzero conductance level, it is convenient in our case to express the matrix Q, sometimes called the microscopic generator of the process, as a sum of two distinct matrices, namely,

$$Q = T + A, \tag{4}$$

where T is a matrix formed by all the elements of Q leading to transitions between distinguishable (i.e., by amplitude) states and A, the matrix that contains all the remaining entries of Q.

These matrices can easily be obtained using the projector matrices Pr^{o} (open) and Pr^{c} (closed) defined as

$$Pr^{e} = \sum_{i \epsilon \{e\}} |\mathrm{s}_i\rangle\langle \mathrm{s}_i|, \ (e = \mathrm{o}, \mathrm{c}) \tag{5}$$

where $\{e\}$ = set of all the open or closed states, $\langle \mathrm{s}_i|$ the ith state row vector given by

$$\langle \mathrm{s}_i| = (\delta_{1i}, \delta_{2i}, \ldots, \delta_{ni}) \tag{5a}$$

with

$$\begin{aligned} \delta_{ji} &= 0 \text{ if } i \neq j \\ &= 1 \text{ if } i = j \end{aligned} \tag{5b}$$

and $|\mathrm{s}_i\rangle = \langle \mathrm{s}_i|^t$ = the associated transposed column vector (see the Appendix for an example of how projector matrices can be calculated). Note that the $\{\bar{e}\}$ will refer to the set of all the open states, if $\{e\}$ is defined as the set of all the closed states and vice versa. The matrices T and A can now be expressed as

$$T = Pr^{\mathrm{o}} Q Pr^{\mathrm{c}} + Pr^{\mathrm{c}} Q Pr^{\mathrm{o}} \tag{6a}$$

and

$$A = Pr^{\mathrm{o}} Q Pr^{\mathrm{o}} + Pr^{\mathrm{c}} Q Pr^{\mathrm{c}}, \tag{6b}$$

with o = open, c = closed. The entry (i, j) of the matrix $e^{A\tau}$ will thus correspond to the probability that the system starts at $t = 0$ in state $i \epsilon \{e\}$ and ends at a time t in state $j \epsilon \{e\}$ without any transitions to one of the states in $\{\bar{e}\}$ in the interim.

Within the framework provided by this formalism, it is possible to show that $p_e(\tau)$, the probability density of having an open (e = o) or closed (e = c) time interval of length $\tau, \tau + \mathrm{d}\tau$ is given by

$$p_e(\tau)\mathrm{d}\tau = \frac{\langle p_{\mathrm{eq}}|\, TPr^{e} e^{A\tau} T|\, \mathrm{U}\rangle}{\langle p_{\mathrm{eq}}|\, TPr^{e}|\, \mathrm{U}\rangle}\, \mathrm{d}\tau \qquad (e = \mathrm{o}, \mathrm{c}) \tag{7}$$

where $\langle p_{\mathrm{eq}}|$ is the steady state solution of Eq. 2 and $|\mathrm{U}\rangle$ is a summation column vector with all its entries equal to 1 (see also Colquhoun and Hawkes, 1981). The expression in Eq. 7 was obtained by considering the conditional probability that a system stays for a period of time t only within the states $\epsilon\{e\}$ (the term $e^{A\tau}$ in Eq. 7) with at $\tau e[\tau, \tau + \mathrm{d}\tau]$ a

[1]Throughout this paper the following notation will be used: row vectors will be represented by $\langle \mathrm{v}_1|$ and column vectors by $|\mathrm{v}\rangle$. The product of a row vector by a column vector will thus be written as $|\mathrm{v}_1\rangle\langle \mathrm{v}_2|$, whereas $\langle \mathrm{v}_1|\mathrm{v}_2\rangle$ will represent the scalar product of v_1 and v_2.

transition from $\{e\}$ to $\{\bar{e}\}$ (the term $T\mathrm{d}\tau$ in Eq. 7), knowing that a transition from one state in $\{\bar{e}\}$ to a state in $\epsilon\{e\}$ took place at $t = 0$ ($\langle p_{eq}|\, T p_{\bar{e}}^{e}|\mathbf{U}\rangle$ in Eq. 7).

Omission Problem: The Exact Solution

The time interval omission problem we intend to resolve is not totally equivalent to the time interval distribution problem of a damped random two-state signal. The latter problem includes, obviously, some aspects of the timer interval omission problem, since very short time intervals will also not be detected due to filtering. A general solution to this problem has not yet been worked out; so far, only the special case of a symmetrical two-state channel has been resolved exactly (Rickard, 1977; see also FitzHugh, 1983, for a discussion of the asymmetrical case).

Here we will be concerned solely with how the open or closed time interval distribution of an ideal signal will be modified due to the omission of time intervals shorter than a minimum value τ_m. What is required in that case is to compute the probability density $p_e(\tau, \tau_m)$, the probability density of having an open (e = o) or closed (e = c) time interval of length $[\tau, \tau + \mathrm{d}\tau]$ for a minimum time interval resolution τ_m.

The probability density $p_e(\tau, \tau_m)$ can be expressed as a conditional probability

$$p_e(\tau, \tau_m)\mathrm{d}\tau = P_e(B_2/B_1)\,, \tag{8}$$

where B_1 is the event after a stay longer than τ_m in the set $\{\bar{e}\}$, there is in the interval $[t_o, t_o, + \mathrm{d}t_o]$ an observable transition to one of the states $\epsilon\{e\}$; $t_o = 0$; and B_2 is the event after t_o, the first observable transition leading to a stay longer than τ_m in the set $\{\bar{e}\}$ occurs at a time τ, $\tau + \mathrm{d}\tau$ (see Fig. 1).

Within the proposed framework, time intervals associated with states in $\{e\}$ and shorter than τ_m will also be taken into account (τ_i, i_{odd} in Fig. 1). Such an approach was chosen since it simplified the computational procedure of $p_e(\tau, \tau_m)$ without invalidating the final conclusions of the present study. It remains nevertheless possible, once $p_e(\tau, \tau_m)$ has been correctly estimated, to use for computational purposes only those intervals in $\{e\}$ longer than τ_m (see Neher and Steinbach [1978] for a discussion of this problem).

It thus follows from the definition of a conditional probability that

$$p_e(\tau, \tau_m)\,\mathrm{d}\tau = \frac{P_e(B_1 \text{ and } B_2)}{P_{\bar{e}}(B_1)}\,, \tag{9}$$

where

$$P_{\bar{e}}(B_1) = \frac{\langle p_{eq}|\, T Pr^{\bar{e}}\, e^{A\tau_m}(-A^{-1})\, T|\mathbf{U}\rangle}{\langle p_{eq}|\, T Pr^{\bar{e}}|\mathbf{U}\rangle}\,. \tag{10}$$

The result in Eq. 10 was obtained by integrating Eq. 7 from τ_m to infinity for $\{\bar{e}\}$.

The event B_2 can be realized in many different ways. Let us define $B_2(n)$ as one particular realization of B_2 in which exactly n stays in the subset $\{\bar{e}\}$ have occurred during the time interval $(0, \tau)$. Thus

$$p_e(\tau, \tau_m)\,\mathrm{d}\tau = \sum_{n=0}^{\infty} \frac{P_e[B_1 \text{ and } B_2(n)]}{P_{\bar{e}}(B_1)} \tag{11}$$

where $P_e\,[B_1 \text{ and } B_2(n)]$ is given by

$$\frac{\langle p_{eq}|\, T Pr^{\bar{e}}\, e^{A\tau_m}(-A^{-1})\; T\, R(n, \tau, \tau_m)\, e^{A\tau_m}(-A^{-1})\, T|\mathbf{U}\rangle}{\langle p_{eq}|\, T Pr^{\bar{e}}|\mathbf{U}\rangle}\,, \tag{12}$$

in which $R\ (n, \tau, \tau_m)$ is a restricted transition matrix expressed as

$$R(n, \tau, \tau_m) = \int \mathrm{d}t_1 \cdots \int \mathrm{d}t_{2n+1} e^{At_1}\, T \cdots T\, e^{At_{2n+1}}\, T \qquad \begin{Bmatrix} \tau \leqq \Sigma\, t_i \leqq \tau + \mathrm{d}\tau \\ t_i \text{ even} \leqq \tau_m \end{Bmatrix}. \tag{13}$$

The element (i, j) of $R\ (n, \tau, \tau_m)$ thus corresponds to the probability that a system starting in state i at $t = 0$ with $i \in \{e\}$ will undergo n transitions into the subset $\{\bar{e}\}$, with each stay in $\{\bar{e}\}$ shorter than τ_m and will end in the interval $[\tau, \tau + \mathrm{d}\tau]$ in state $j, j\epsilon\{\bar{e}\}$. The constraint $\tau \leqq \Sigma\tau_i \leqq \tau + \mathrm{d}\tau$ can be taken into account by using a Dirac delta function, namely,

$$\delta(\tau - \Sigma\, t_i)\mathrm{d}\tau = \frac{1}{2\pi}\int_{-\infty}^{\infty} e^{iu(\Sigma t_i - \tau)}\,\mathrm{d}u\,\mathrm{d}\tau\,. \tag{14}$$

The restriction on τ_i even $\leqq \tau_m$ can be directly included in the integral Eq. 13. It thus follows that Eq. 13 can be written as

$$\mathbf{R}(n, \tau, \tau_m) = \frac{1}{2\pi}\int_{-\infty}^{\infty} e^{-iu\tau}\int_0^{\infty} e^{(A+\mathbf{I}iu)t}\,\mathrm{d}t\; T \left[\int_0^{\tau_m} e^{(A+\mathbf{I}iu)t}\,\mathrm{d}t\; T \int_0^{\infty} e^{(A+\mathbf{I}iu)t}\,\mathrm{d}t\; T\right]^n \mathrm{d}u\,\mathrm{d}\tau\,, \tag{15}$$

where $\mathbf{I}$ is the identity matrix. Eq. 15 may now be incorporated into the summation term in Eq. 11, and the probability density $p_e(\tau, \tau_m)$ reads after lengthly algebra

$$p_e(\tau, \tau_m) = \langle \text{initial}_e|\, F(\tau, \tau_m)\,|\text{final}\rangle \tag{16a}$$

where

$$\langle \text{initial}_e| = \frac{\langle p_{eq}|\, T Pr^{\bar{e}}\, e^{A\tau_m}(-A^{-1})\; T}{\langle p_{eq}|\, T Pr^{\bar{e}}\, e^{A\tau_m}(-A^{-1})\; T|\mathbf{U}\rangle} \tag{16b}$$

$$F(\tau, \tau_m) = \frac{1}{2\pi}\int_{-\infty}^{\infty} e^{-iu\tau} - (A + iu\mathbf{I})^{-1}\; T \cdot \left[\mathbf{I} - (\mathbf{I} - e^{(A+iu\mathbf{I})\tau_m})(A + iu\mathbf{I})^{-1}\; T\,(A + iu\mathbf{I})^{-1}\; T\right]^{-1} \mathrm{d}u\,, \tag{16c}$$

and

$$|\text{final}\rangle = e^{A\tau_m}(-A^{-1})\,T|U\rangle\,. \tag{16d}$$

Eqs. 16 constitute the general solution to the omission problem. However, the matrix function $F(\tau, \tau_m)$ needs, even for the simplest cases, to be computed numerically. A fast Fourier transform (FFT) program can be used for that particular purpose, but the computational feasibility of this integral formalism remains, nevertheless, quite limited.

First-order Approximation

Let us consider the more restrictive case in which $\Sigma\, t_i$ even $\ll \Sigma t_{iodd}$ (see Fig. 1). Physically, this inequality implies that the mean lifetime of the gaps is much smaller than the mean value of the time intervals in $\{e\}$. Under this condition

$$\delta(\tau - \Sigma\, t_i) \simeq \delta(\tau - \Sigma\, t_{iodd}) \tag{17}$$

and the function $F(\tau, \tau_m)$ in Eq. 16c reduces, by using the equality

$$e^{M\tau} = -\frac{1}{2\pi}\int_{-\infty}^{\infty} e^{-iu\tau}\,(M + iu\mathbf{I})^{-1}\,du \tag{18}$$

to a more tractable expression, namely

$$\tilde{F}(\tau, \tau_m) = e^{M\tau}\,T,$$

where

$$M = A + T(\mathbf{I} - e^{A\tau_m})(-A^{-1})\,T. \tag{19}$$

The probability density $p_e(\tau, \tau_m)$ can now be written as

$$\tilde{p}_e(\tau, \tau_m) = \langle \text{initial}_e|\,\tilde{F}(\tau, \tau_m)\,|\text{final}\rangle\,, \tag{20}$$

where $\tilde{F}(\tau, \tau_m)$ is now given by Eq. 19.

Although the Eqs. 16b, 16d, and 20 appear as complex expressions, the computational procedure involved in each case consists merely in multiplying matrices and vectors. Expressions such as $e^{A\tau_m}$ and $e^{M\tau_m}$ can be calculated furthermore using standard methods of linear algebra (see for instance Moler and Van Loan, 1978).

The exponent in Eq. 19 has several interesting properties. It should first be clear that for a critical time $\tau_m = 0$ the matrix M becomes equal to A. Under this condition, Eq. 20 reduces to the expression in Eq. 7 as it should be expected. For $\tau_m \neq 0$ new transitions that were previously forbidden can now occur. This particular aspect is introduced by the operator $-T(\mathbf{I} - e^{A\tau_m})(A^{-1})\,T$, where $(\mathbf{I} - e^{A\tau_m})$ is simply the probability operator of having an interval smaller than τ_m. An analysis in which τ_m is not explicitly taken into account may thus lead to erroneous conclusions since a systematic omission of time intervals introduces new transition rates that connect states that were previously disconnected. This problem can be avoided, however, by using the operator M instead of A while computing the time interval distribution for a particular kinetic scheme.

Examples of Analysis

Let us consider as examples two special kinetic schemes commonly found in single channel studies. The simplest kinetic model one can use to describe the stochastic behavior of a single ionic channel remains the two state open-closed kinetic model, namely,

$$C \underset{K_2}{\overset{K_1}{\rightleftharpoons}} 0. \tag{21}$$

The exact solution for $p_e(\tau, \tau_m)$ obtained from 16a reads in this particular case

$$p_e(\tau, \tau_m) = e^{-K_2\tau_m}\frac{1}{2\pi}\int_{-\infty}^{\infty} e^{-iu\tau}\,\frac{a(u)}{[1 - a(u)\,b(u)]}\,du \tag{22a}$$

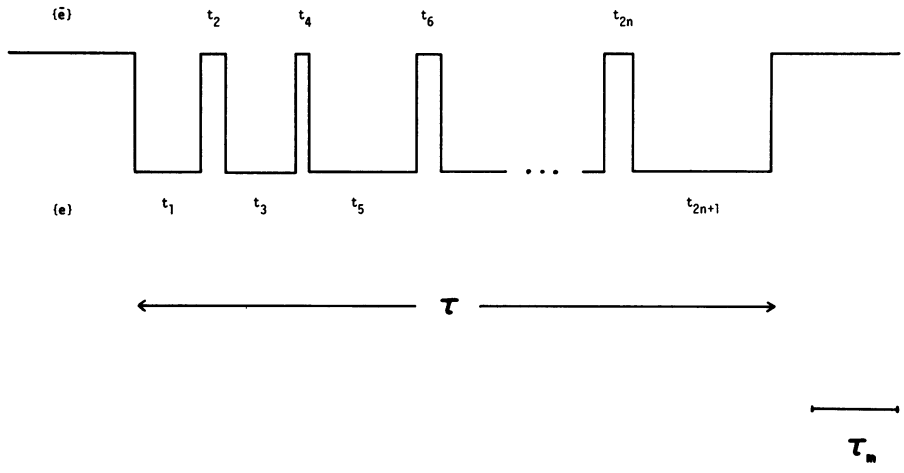

FIGURE 1 Schematic drawing of a random two state signal with brief intervals in $\{\bar{e}\}$. The time intervals $t_2, t_4 \ldots t_{2n}$, refer to time gaps of length smaller than the critical time, τ_m. The resulting time interval in $\{e\}$ will be equal to τ.

with

$$a(u) = \frac{K_1}{(K_1 - iu)}$$

$$b(u) = \frac{K_2}{(K_2 - iu)}(1 - e^{-(K_2 - iu)\tau_m}) . \qquad (22b)$$

The probability density $p_o(\tau, \tau_m)$ can easily be derived from Eq. 22a by substituting K_1 by K_2 and vice versa. We show in Fig. 2 the results of numerical calculations in which Eq. 22a was computed using an FFT algorithm. The value of K_1 and K_2 was set to 1 in this particular case and $p_c(\tau, \tau_m)$ was estimated for values of τ_m ranging from 0 to 1.0. As seen, an increase in τ_m results, for time intervals >2.0, in an overestimation of the time interval probability density. This corresponds to the expected behavior of a random signal in which short time intervals have been systematically omitted. We note also that the time interval probability density for times smaller than τ_m is not equal to zero. This particular point arises from the approach we used to compute probability density $p_e(\tau, \tau_m)$, the restriction in Eq. 13 being applied only to t_{ieven}, the time intervals of the gaps. Such a procedure does not rule out that t_{iodd} may be smaller than τ_m, and thus predicts a nonzero

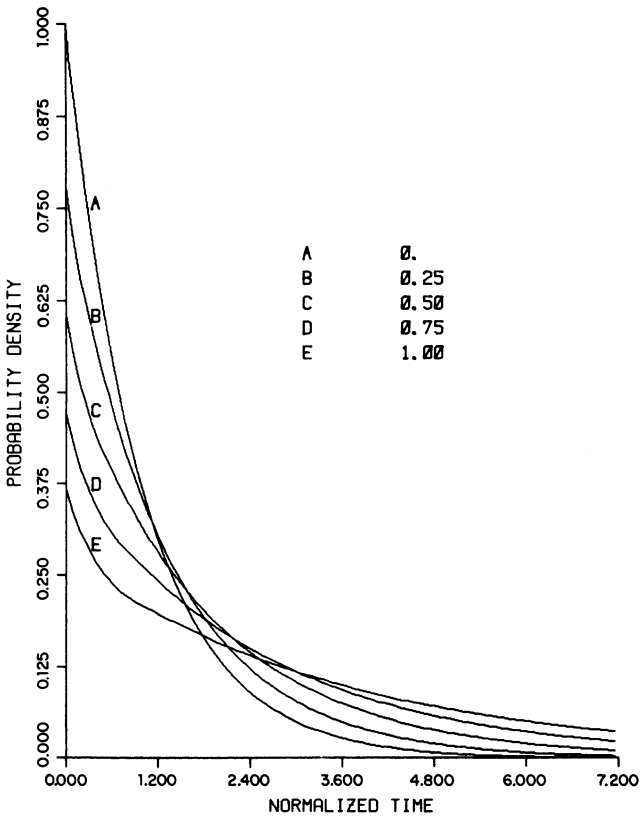

FIGURE 2 Results of numerical calculations in which the exact solution (Eq. 22) for a two state model was computed using an FFT algorithm. The time constants K_1 and K_2 were set to 1 s^{-1}. The value of the critical time τ_m was varied from 0 to 1 s. An increase in the probability density for normalized times ($K\tau_m$) greater than 2 can be observed as the value of τ_m is increased.

probability density for the t_{iodd} intervals (see Fig. 1). In cases where τ_m is comparable to or greater than K_1^{-1} or K_2^{-1}, it should also be apparent that the probability density $p_e(\tau, \tau_m)$ does not remain a single exponential function (see Fig. 1 *e*). This particular point is especially important since it may lead to an erroneous conclusion concerning the total number of states to be associated with a time interval distribution function $p_e(\tau, \tau_m)$. This problem can, however, be avoided by taking only values of τ greater than τ_m. A more useful expression can be obtained using the approximate formulation proposed in Eq. 19. It may then be shown that (see Appendix I) the probability density $\tilde{p}_c(\tau, \tau_m)$ reduces to

$$\tilde{p}_c(\tau, \tau_m) = K_1 e^{-K_2\tau_m} e^{-(K_1 e^{-K_2\tau_m})\tau} \qquad (23)$$

with $\tilde{p}_o(\tau, \tau_m) = \tilde{p}_c(\tau, \tau_m) : K_1 \leftrightarrow K_2$.

As seen when $\tau_m = 0$, $\tilde{p}_c(\tau, 0) = K_1 e^{-K_1\tau}$, which is a standard result for the kinetic scheme proposed in Eq. 21. It should also be clear from Eq. 23 that $\tilde{p}_c(\tau, \tau_m)$ and $\tilde{p}_o(\tau, \tau_m)$ will remain single exponential functions as long as the approximation in Eq. 17 is valid. The values of K_1 and K_2 can, in principle, be obtained from $\tilde{p}_c(\tau, \tau_m)$ and $\tilde{p}_o(\tau, \tau_m)$ by computing for various values of τ_m the semilogarithm slope of $\tilde{p}_e(\tau, \tau_m)$, ($e$ = o, c) vs. τ_m.

Similar expressions have already been reported in the literature using a different mathematical approach (Neher, 1983; Sachs and Auerbach, 1983). The expression proposed by Neher (1983) can be reconciled, if one takes into account in our computational procedure the mean duration of the interruptions. This is in essence equivalent to replacing Eq. 17 by

$$\delta(\tau - \Sigma t_i) \simeq \frac{1}{2\pi}\int_{-\infty}^{\infty} du\, e^{-iu(\tau - \Sigma t_{iodd})}\left[\prod_{k=1}^{n}(1 + iut_{2k})\right], \qquad (24)$$

where $e^{iut_{even}}$ has been approximated by $(1 + iut_{even})$. The first-order terms will yield directly the expression proposed in (23), whereas keeping the second-order terms will lead to the results found by Neher (1983) for the two-state model. It will be shown by comparing the exact and approximate solutions for a more complex kinetic scheme that the approximation proposed in this work should, for most experimental cases, be adequate.

Let us consider as an example of a more elaborate kinetic scheme the following model:

$$C \underset{K_2}{\overset{K_1}{\rightleftharpoons}} O \underset{K_4}{\overset{K_3}{\rightleftharpoons}} C \qquad (25)$$

Detailed calculations presented in Appendix show that within the limits provided by approximation discussed previously, the probability densities $\tilde{p}_o(\tau, \tau_m)$ and $\tilde{p}_c(\tau, \tau_m)$ now read

$$\tilde{p}_o(\tau, \tau_m) = -\lambda_0 e^{\lambda_0} \qquad (26a)$$

$$\tilde{p}_c(\tau, \tau_m) = \xi\left[\frac{K_1 K_4 + \lambda_1 \overline{K}}{(\lambda_1 - \lambda_2)} e^{\lambda_1\tau} + \frac{K_1 K_4 + \lambda_2 \overline{K}}{(\lambda_2 - \lambda_1)} e^{\lambda_2\tau}\right] \qquad (26b)$$

where

$$\lambda_0 = -(K_2 e^{-K_1\tau_m} + K_3 e^{-K_4\tau_m}) \tag{26c}$$

$$\lambda_{1/2} = \frac{1}{2}\Big(-[K_1 + K_4 + (\xi - 1)\bar{K}] \pm \{[K_1 + K_4 + (\xi - 1)\bar{K}]^2 - 4K_1K_4\xi\}^{1/2}\Big) \tag{26d}$$

$$\bar{K} = K_1 \frac{K_2}{K_2 + K_3} + K_4 \frac{K_3}{K_2 + K_3} \tag{26e}$$

and

$$\xi = e^{-(K_2+K_3)\tau_m}. \tag{26f}$$

The results obtained in Eqs. 26a and 26b illustrate again more clearly that the omission of time intervals shorter than τ_m does not lead, as long as the limits expressed in Eq. 17 are valid, to time interval distribution functions containing additional time constants not present in the standard formalism obtained by assuming $\tau_m = 0$. The number of states related to a particular kinetic scheme can thus be always correctly estimated within the limits provided by Eq. 17. However, the time constants will be substantially modified and a correct evaluation of the rate constants does not appear to be possible without taking into account explicitly the effect of τ_m on $\tilde{p}_c(\tau, \tau_m)$ or $\tilde{p}_o(\tau, \tau_m)$. If one assumes furthermore that the value of τ_m is selected so that $K_4\tau_m \gg 1$ and $K_1\tau_m \ll 1$, then the resulting probability

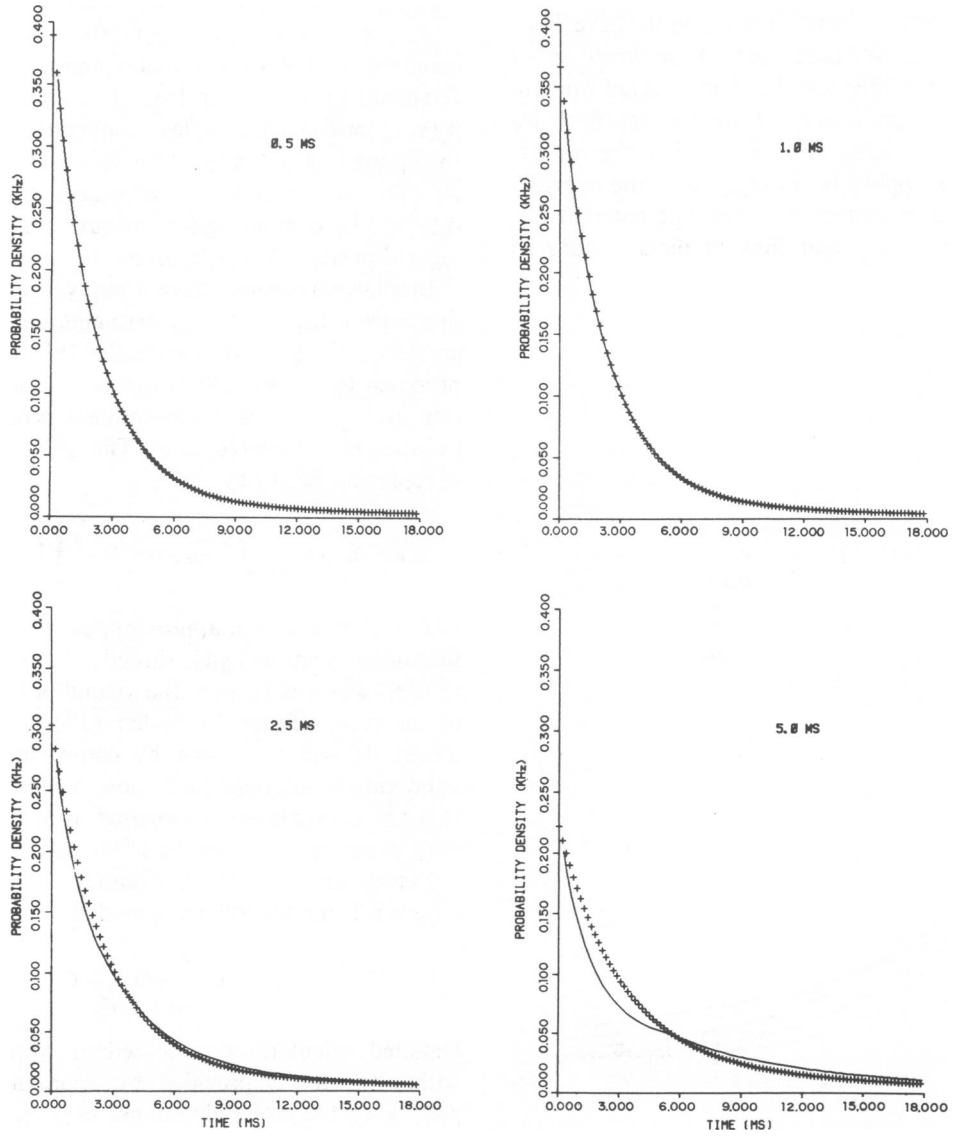

FIGURE 3 Comparison between the exact (continuous line) and approximate (discontinuous line) solution for the closed time interval distribution $p_c(\tau, \tau_m)$ for the three-state model C-O-C. The value of $p_c(\tau, \tau_m)$ was computed using Eq. 16a in one case and Eq. 26b in the other. The value of K_1, K_2, K_3, and K_4 were equal to 75, 25, 100, and 500 s^{-1}, respectively. The value of τ_m was varied from 0 to 5 ms. Both solutions were found to be equivalent for values of τ_m smaller than 2 ms.

density for the open state intervals becomes

$$\tilde{p}_o(\tau, \tau_m) \simeq K_2 e^{-K_2\tau}. \quad (27)$$

Under such conditions, it is thus possible by means of a half amplitude minimum time interval procedure to systematically remove the "flickering" component of a random signal from the slower kinetic pathways. It should also be clear from the Eq. 26c that the four rate constants K_1, K_2, K_3, and K_4 can be obtained by considering the variations of λ_0 as a function of τ_m. The value of λ_0 can be easily measured since the open time interval distribution in this particular case corresponds to a single exponential function. The solution expressed in Eqs. 26a–26f was obtained assuming the condition in Eq. 17. In Fig. 3 we compare the exact solution of $p_c(\tau, \tau_m)$ as computed from Eq. 16a for the C-O-C model and the approximate solution proposed in Eq. 26b. As seen both solutions will agree rather well for values of τ_m smaller than 2.5 ms. This result is interesting since the value of $1/K_3$ and $1/K_4$ in this particular case were equal to 10 and 2 ms, respectively. It thus appears that the approximate formalism proposed in Eq. 20 can be applied even if the rate constants involved are comparable to $1/\tau_m$.

Application of the Proposed Formalism to the Damped Random Signal Problem

We mentioned previously that the time interval omission problem is not totally equivalent to the time interval distribution problem of a damped random signal. Additional effects, such as the finite rise time of the transitions between open and closed states, must be included. Since filtering procedures are common in patch-clamp experiments, numerical calculations were undertaken to determine how well the approximate formula proposed in Eq. 19 could be used to describe the effect of filtering on the open or closed time interval distribution of a patch clamp signal. An exact solution to the damped random signal problem exists only for the symmetrical open-closed kinetic scheme (Rickard, 1977). The formal solution proposed by Rickard has the following form:

$$p_o(\tau) = \sum_{j=1}^{\infty} q(\sigma_j)\, e^{2K\sigma_j\tau/\alpha}, \quad (28a)$$

where

$$q(\sigma_j) = \frac{4K}{\alpha}\left[\psi(\sigma_j) - \psi(\sigma_j + \alpha) + \psi\left(\sigma_j + \alpha + \frac{1}{2}\right) - \psi\left(\sigma_j + \frac{1}{2}\right)\right]^{-1} \quad (28b)$$

$$\psi(x) = \frac{d}{dx}\ln[\Gamma(x)] \quad (28c)$$

for K the transition rate and $\alpha = KRC$, where RC is the time constant of the filter and σ_i the roots of

$$\Gamma(\sigma_j)\,\Gamma\left(\sigma_j + \alpha + \frac{1}{2}\right) + \Gamma\left(\sigma_j + \frac{1}{2}\right)\Gamma(\sigma_j + \alpha) = 0, \quad (28d)$$

where Γ refers to the usual gamma function. As expressed in Eq. 28a, the time interval distribution $p_o(\tau)$ corresponds to a sum of exponential functions.

We present in Fig. 4 *a* numerical evaluation of Eq. 28a for various value of α. In general, for $t \gg RC$ the resulting curves decline exponentially as one of the exponential term in Eq. 28a becomes more dominant. It should be obvious that the approximate solution for the open-closed model expressed in Eq. 23 cannot properly describe the time interval probability density for time intervals shorter than RC. If, however, one compares the dominant exponential decay shown in Fig. 4 with the prediction of Eq. 23 for $\tau_m = RC\ \ln(2)$, the resulting time constants will be almost identical as shown in Fig. 5. In this figure the value of the time constant of the exponential decay shown in Fig. 4 was estimated for various values of α with $K = 1$. This time constant was then compared to $K' = (1/2)^{\alpha}$, which was

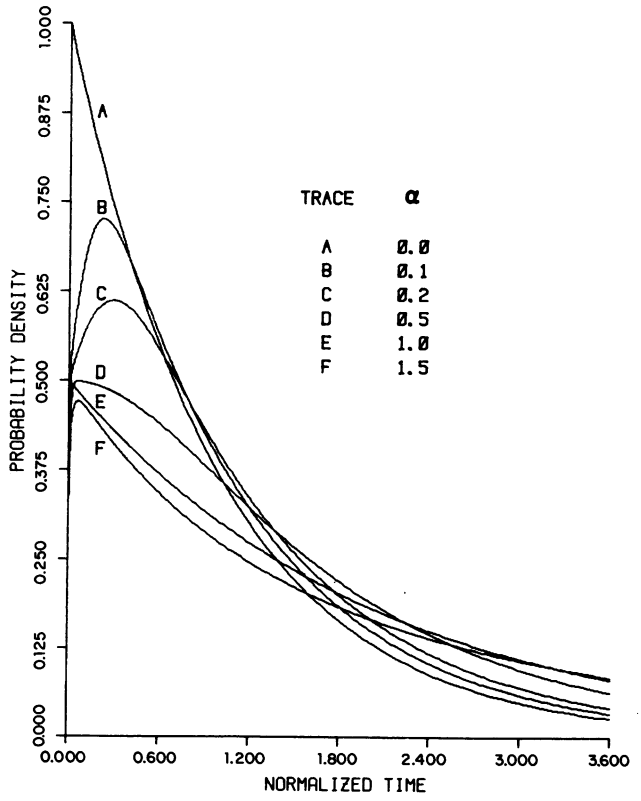

FIGURE 4 Exact solution for the time interval distribution of a damped symmetrical two state random signal. The parameter α is equal to KRC where K is the rate of transitions and RC the time response of the filter. Calculations were carried for $K = 1\ s^{-1}$. The normalized time corresponds to $K\tau$ where τ is the length of the time interval. As seen for normalized times > 1, the probability density decreases exponentially. This portion of curves can be reproduced using the approximate solution we found for the time interval distribution of a two-state signal (Eq. 23).

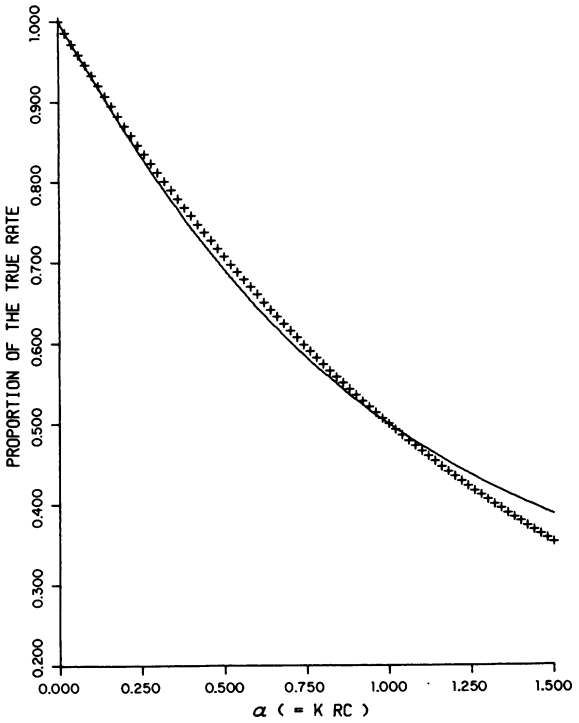

FIGURE 5 Comparison between the rate of transitions as determined from the exponential decay of the exact solution shown in Fig. 4, and that predicted from Eq. 23 with $K_1 = K_2 = 1$ and $\tau_m = RC \ln(2)$. The true rate should be equal to 1. As the time of response (RC) of the filter is increased, the apparent rate computed from the exponential decay shown in Fig. 4 decreases. The approximate solution Eq. 23 predicts that the apparent rate should be equal to $(\frac{1}{2})^{\alpha}$, which is closed to the exact solution for values of $\alpha < 1.2$.

obtained from Eq. 23 with τ_m corresponding to the critical time for half amplitude [$\tau_m = RC \ln(2)$] and $K_1 = K_2 = 1$. Interestingly, the approximate formulation proposed in Eq. 23 for the simple two state model constitutes an excellent asymptotic solution to the damped random signal problem. The half-amplitude minimum-interval criterion is thus like to be a valuable approach to take into account the effect of filtering on the time interval distribution of patch clamp signals. By using the general framework proposed in this work with $\tau_m = RC \ln(2)$, one can thus take into account explicitly the effect of filtering on $p_o(\tau, \tau_m)$ and $p_c(\tau, \tau_m)$ for any particular kinetic scheme.

CONCLUSION

The main purpose of this work was to present a general solution to the time interval omission problem. The main result of our analysis is expressed in Eqs. 16a through 16d and in an approximate form in Eq. 20. The latter formulation does not require the evaluation of the integral in Eq. 16c and can easily be implemented in a standard curve-fitting procedure. In this regard, the equations associated with a specific kinetic scheme can explicitly include the minimum time interval τ_m and thus describe more accurately the time interval distribution measured experimentally. This procedure does not result in additional curve-fitting parameters but in a more elaborate mathematical form for the time interval probability density. It was also shown that a more accurate estimate of the transition rates associated with a given kinetic scheme could be obtained through an analysis procedure focused mainly on how the different experimentally measured time constants vary as a function of τ_m. The formalism proposed here can thus be regarded as a general tool by means of which analytic expressions relating each time constant to τ_m can be derived. These expressions can afterwards be used in a curve fitting procedure to obtain transition rate values. The proposed formalism should thus lead to a better discrimination among equivalent kinetic schemes by allowing more precise estimates of the transition rates.

Although the general solution we propose was derived without including directly the effect of damping on the time interval distribution, we found by comparing the exact solution of Rickard (1977) for the symmetrical open-closed model to the approximate solution we obtained for this particular kinetic scheme, that our formalism can serve, with τ_m given by $RC \ln(2)$, as an asymptotic approximation by means of which the time interval distribution of a damped signal can be expressed. This also represents an improvement over the prevailing theories on damped random signals, since the formula that we derived can be applied to any kinetic scheme with a single nonzero conducting state. It is worth mentioning in this regard that the general mathematical framework discussed here can easily be extended by introducing specific projectors to multiple nonzero conducting state kinetic schemes, each projector being associated with a given conductance level.

Finally, it was shown that the omission of time intervals did not lead to mathematical expressions for $p_e(\tau, \tau_m)$ and $p_o(\tau, \tau_m)$ containing additional time constants, as long as the approximation expressed in Eq. 17 was satisfied. It was also concluded that an analysis of open or closed time interval distributions based on a formalism in which τ_m is not explicitly included may result in erroneous conclusions, since one consequence of using a time interval criterion was to connect channel states that were previously disconnected (Eq. 19). This problem may, however, be circumvented through an analysis of the effect of τ_m on the time interval distributions measured experimentally. The minimum time interval criterion appears then as a computational procedure through which distinct kinetic pathways can be isolated and thus analyzed. By taking into account the effect of τ_m, the proposed formalism serves therefore as a general tool for single-channel analysis.

APPENDIX

We present in the Appendix the detailed calculations of $p_o(\tau, \tau_m)$ and $p_c(\tau, \tau_m)$ for the three-state kinetic scheme

$$C \underset{K_2}{\overset{K_1}{\rightleftharpoons}} O \underset{K_4}{\overset{K_3}{\rightleftharpoons}} C. \qquad \text{(A1)}$$

The solution for the two-state model can be derived by following a similar procedure. To obtain for $p_o(\tau, \tau_m)$ and $p_c(\tau, \tau_m)$ closed form solutions, we will use the approximate Eq. 20. For the three-state models presented in Eq. A1, the microscopic generator matrix **Q** reads

$$\mathbf{Q} = \begin{bmatrix} -(K_2 + K_3) & K_2 & K_3 \\ K_1 & -K_1 & 0 \\ K_4 & 0 & -K_4 \end{bmatrix} \tag{A2}$$

and the vector $\langle s_1|$, $\langle s_2|$ and $\langle s_3|$ are given by

$$\begin{aligned} \langle s_1| &= (1, 0, 0) \\ \langle s_2| &= (0, 1, 0) \\ \langle s_3| &= (0, 0, 1) \end{aligned} \tag{A3}$$

and

$$\langle U| = (1, 1, 1).$$

The projector matrices Pr^c and Pr^o can be obtained by substituting the vector $\langle s_1|$, $\langle s_2|$, and $\langle s_3|$ in Eq. 5. The final result is

$$\begin{aligned} Pr^o &= \begin{bmatrix} 1 & 0 & 0 \\ 0 & 0 & 0 \\ 0 & 0 & 0 \end{bmatrix} \\ Pr^c\ (e = o, c) &= \begin{bmatrix} 0 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix}. \end{aligned} \tag{A4}$$

The matrices A and T can now be computed uisng the Eqs. 6a and 6b. A simple calculation gives

$$A = \begin{bmatrix} -(K_2 + K_3) & 0 & 0 \\ 0 & -K_1 & 0 \\ 0 & 0 & -K_4 \end{bmatrix} \tag{A5a}$$

and

$$T = \begin{bmatrix} 0 & K_2 & K_3 \\ K_1 & 0 & 0 \\ K_4 & 0 & 0 \end{bmatrix}. \tag{A5b}$$

Since the matrix A has all its nondiagonal entries equal to zero, it can be easily shown that the operator $e^{A\tau_m}$ reduces in that case to

$$\begin{bmatrix} e^{-(K_2+K_3)\tau_m} & 0 & 0 \\ 0 & e^{-K_1\tau_m} & 0 \\ 0 & 0 & e^{-K_4\tau_m} \end{bmatrix}. \tag{A6}$$

The matrix M defined as $M = A + T(\mathbf{1} - e^{A\tau_m})(-A^{-1})\,T$ can now be directly computed. Lengthly algebra leads to

$$\mathbf{M} = \begin{bmatrix} -(K_2 e^{-K_1\tau_m} + K_3 e^{-K_4\tau_m}) & 0 & 0 \\ 0 & \left[\frac{K_2}{K_2 + K_3}(1 - \xi) - 1\right] K_1 & \frac{K_3}{K_2 + K_3}(1 - \xi) K_1 \\ 0 & \frac{K_2}{K_2 + K_3}(1 - \xi) K_4 & \left[\frac{K_3}{K_2 + K_3}(1 - \xi) - 1\right] K_4 \end{bmatrix}, \tag{A7}$$

where

$$\xi = e^{-(K_2+K_3)\tau_m}.$$

To compute the operator $e^{M\tau}$, it is advantageous to use the expression in Eq. 18. One needs in that case to invert the matrix $(M + iu\mathbf{l})$ and to integrate each entry of the resulting matrix. It can be shown that the operator $\tilde{F}(\tau, \tau_m)$ defined as $e^{M\tau}T$ is given then by

$$\tilde{F}(\tau, \tau_m) = \begin{bmatrix} 0 & K_2 e^{-P_c(K_2+K_3)\tau} & K_3 e^{-P_c(K_2+K_3)\tau} \\ \dfrac{K_1(K_4 + \lambda_1)e^{\lambda_1\tau} - K_1(K_4 + \lambda_2)e^{\lambda_2\tau}}{(\lambda_1 - \lambda_2)} & 0 & 0 \\ \dfrac{K_4(K_1 + \lambda_1)e^{\lambda_1\tau} - K_4(K_1 + \lambda_2)e^{\lambda_2\tau}}{(\lambda_1 - \lambda_2)} & 0 & 0 \end{bmatrix}, \tag{A8}$$

with λ_0, λ_1, and λ_2 are the roots of

$$(\lambda - \lambda_0)\{\lambda^2 + [K_1 + K_4 + \bar{K}(\xi - 1)]\lambda + K_1 K_4 \xi\} = 0 \tag{A9}$$

and

$$P_c = \frac{1}{K_2 + K_3}(K_2 e^{-K_1\tau_m} + K_3 e^{-K_4\tau_m}).$$

The final expression for $p_c(\tau, \tau_m)$ can now be obtained from Eq. 20 with

$$\begin{aligned} \langle \text{initial}_{\text{open}}| &= (1, 0, 0) \\ \langle \text{initial}_{\text{closed}}| &= (0, K_2, K_3)\frac{1}{K_2 + K_3} \end{aligned} \tag{A10}$$

$$|\text{final}\rangle = \begin{bmatrix} e^{-(K_2+K_3)\tau_m} \\ e^{-K_1\tau_m} \\ e^{-K_4\tau_m} \end{bmatrix}. \tag{A11}$$

This work was supported by the Medical Research Council of Canada and by the Fonds de la Recherche en Santé du Québec.

Received for publication 23 August 1984 and in final form 21 February 1985.

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