SINGLE-CHANNEL AUTOCORRELATION FUNCTIONS: THE EFFECTS OF TIME INTERVAL OMISSION

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ABSTRACT We present a general mathematical framework for analyzing the dynamic aspects of single channel kinetics incorporating time interval omission. An algorithm for computing model autocorrelation functions, incorporating time interval omission, is described. We show, under quite general conditions, that the form of these autocorrelations is identical to that which would be obtained if time interval omission was absent. We also show, again under quite general conditions, that zero correlations are necessarily a consequence of the underlying gating mechanism and not an artefact of time interval omission. The theory is illustrated by a numerical study of an allosteric model for the gating mechanism of the locust muscle glutamate receptor-channel.

INTRODUCTION

Single-channel recording is now established as the method of choice for investigating the kinetics of receptor gating of ion channels (Auerbach and Sachs, 1984; Horn, 1984). The principal aim of such studies is to obtain a complete kinetic description of the channel-gating process, with a view to being able to relate such a description to the underlying molecular events. The later stages of such investigations involve objective estimation of the kinetic parameters of a particular gating mechanism (Horn and Lange, 1983). However, before such an analysis is possible, it is necessary to establish the overall form of the gating mechanism. That is, one must determine the number of open states and the number of closed states accessible to the channel, and also the number of isomerisation pathways which link the open states with the closed.

Methods for determining the number of channel states via analysis of channel sojourn time pdfs (Colquhoun and Sigworth, 1983) have been applied to a variety of systems. However, it is only recently that the theoretical background to methods for determining the number of channel isomerisation pathways has been developed. These methods (Jackson et al., 1983; Fredkin et al., 1985; McManus et al., 1985) all rely on some form of analysis of correlations between successive channel sojourn times. The use of sojourn time autocorrelation functions (Fredkin et al., 1985; Colquhoun and Hawkes, 1987) is particularly attractive as it allows for the possibility of establishing a lower bound to the number of isomerisation pathways (m). Briefly, if there is only a single pathway linking the open states with the closed states ($m = 1$), then a null autocorrelation function is observed. If $m > 1$, as in branched or cyclic gating mechanisms, then the observed autocorrelation function is made up of $\leq m - 1$ geometrically decaying terms. Analysis of channel sojourn time autocorrelation functions has been applied to the nicotinic acetylcholine receptor by Labarca et al. (1985), and to the locust muscle glutamate receptor by Kerry et al. (1987*a*, *b*). In both cases significant autocorrelations were seen, indicative of branched or cyclic gating mechanisms for these receptor channels.

There is, however, a complication when attempting to relate observed channel kinetics to those predicted on the basis of these simple stochastic models. There is a deficit of brief (<0.2 ms) channel sojourn times in experimentally derived data. Such time interval omission arises from the filtering of the data during recording combined with the use of threshold crossing algorithms for event detection during subsequent processing of the raw data. Several authors have analyzed the effect of time interval omission on channel sojourn time pdfs (Roux and Sauvé, 1985; Blatz and Magleby, 1986), and hence on the use of pdfs to estimate the number of kinetic states of a channel. However, there remains a need for a general theory which will also permit analysis of the effects of time interval omission on sojourn time autocorrelation functions. Clearly this is a matter of some importance if analysis of autocorrelation functions is to be used to draw inferences about the number of channel isomerisation pathways.

Here we present a general theory for predicting the effect of time interval omission on single channel kinetics, with particular emphasis on the effects on sojourn time autocorrelation functions. We examine in some detail this approach as applied to a gating model which has proved useful in understanding the kinetics of the glutamate receptor channel (Kerry et al., 1987*a*, *b*).

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METHODS

Calculation of predicted autocorrelations were performed on a Vax 11/780. Simulations and all other calculations were carried out on a Masscomp 5500. All programs were written in Fortran 77, and employed the NAG library of numerical subroutines. The simulation algorithm was that described by Clay and DeFelice (1983), modified for event omission.

GENERAL THEORY

A single channel gating mechanism is modeled by a finite state space, continuous time, Markov chain. Label the states $1, 2, \ldots, n$. Let $O = 1, 2, \ldots, n_o$ and $C = n_o + 1, n_o + 2, \ldots, n$ be the open and closed states respectively. Let n_c be the number of closed states, so that $n_o + n_c = n$. Denote the above Markov chain by $\{X(t);\ t \geq 0\}$, where $X(t)$ is the state that the channel is in at time t. The process $\{X(t);\ t \geq 0\}$ will be time reversible (Colquhoun and Hawkes, 1983), time homogeneous and irreducible.

For $i \neq j$ let q_{ij} be the transition rate of $\{X(t); t \geq 0\}$ from state i to state j. Let Q be the $n \times n$ matrix with off-diagonal elements q_{ij} and diagonal elements $q_{ii} = -\Sigma_{j \neq i}\, q_{ij}$. Partition the matrix Q into

$$Q = \begin{bmatrix} Q_{oo} & Q_{oc} \\ Q_{co} & Q_{cc} \end{bmatrix},$$

where, for example, Q_{oo} corresponds to transitions that remain within the open states. Also, following Roux and Sauvé (1985), it is convenient to define the $n \times n$ matrices Q_0 and Q_1 by

$$Q_0 = \begin{bmatrix} Q_{oo} & 0 \\ 0 & Q_{cc} \end{bmatrix}$$

and

$$Q_1 = \begin{bmatrix} 0 & Q_{oc} \\ Q_{co} & 0 \end{bmatrix}.$$

Thus Q_0 is the matrix of transition rates for transitions within the open or closed states, and Q_1 is the matrix of transition rates for transitions between the open and closed states.

The process $\{X(t);\ t \geq 0\}$ will possess an equilibrium distribution, $\pi = (\pi_1, \pi_2, \ldots, \pi_n)^T$ say, where T denotes transpose. For $i = 1, 2, \ldots, n$, π_i is the (nonzero) equilibrium probability that the channel is in state i. A consequence of reversibility is that the detailed balance conditions

$$\pi_i q_{ij} = \pi_j\, q_{ji}\ (i, j = 1, 2, \ldots, n) \qquad (1)$$

are satisfied (see e.g., Kelly, 1979).

Time Interval Omission

In practice short sojourns in either the open or closed states may not be detected. We shall assume that a sojourn in the open (closed) states is detected if and only if it is greater than some critical length τ_0. A mathematical framework for analyzing the effects of the above form of time interval omission on a single observed sojourn was formalized by Roux and Sauvé (1985). Here we outline a mathematical framework for analysing the effects of the above form of time interval omission on the dynamic stochastic properties of a sequence of observed sojourns comprising a single channel record. This framework enables us to derive expressions for the autocorrelation functions of Fredkin et al. (1985) but now incorporating time interval omission. The above form of time interval omission is assumed for ease of exposition, although it should be noted that it corresponds to the imposition of a consistent minimum sojourn time, as suggested by e.g., Colquhoun and Sigworth (1983). The following may be readily modified to incorporate (*a*) different values of τ_0 for open and closed sojourns, and (*b*) a specified distribution for τ_0, as in Kerry et al. (1987*a*). The mathematical details for the more general case may be found in Ball and Sansom (1988), where formal proofs are provided.

Suppose that the channel, described by the process $\{X(t);\ t \geq 0\}$, is detected as being in an open state at time $t = 0$ and that $X(0) = i$, where $i \in O$. We assume that the channel continues to be detected as being in an open state until there has been a sojourn of length τ_0 in the closed states, at which point the channel becomes detected as being in a closed state. Let T'_1 be the time at which this happens. Thus T'_1 is the minimum value of t for which the channel is in the closed states throughout the interval from $t - \tau_0$ to t. The channel is now detected as being in a closed state and will remain as such until there has been a sojourn of length τ_0 in the open states, at which point the channel is detected again as being in an open state. Let T'_2 be the time at which this happens, so that T'_2 is the minimum value of t greater than T'_1 for which the channel is in the open states throughout the interval from $t - \tau_0$ to t. Continue the process *ad infinitum* to obtain a sequence of times T'_1, T'_2, ... at which channel openings or closings are detected. Set $T'_0 = 0$ and let

$$J_k = X(T'_k) \quad (k = 0, 1, \ldots)$$

and

$$T_k = T'_k - T'_{k-1} \quad (k = 1, 2, \ldots).$$

Thus J_k is the state that the channel is in when the kth opening/closing is detected and T_k is the length of the kth observed sojourn.

The process $\{J_k;\ k = 0, 1, \ldots\}$ is a Markov chain, with period 2, on the state space $\{1, 2, \ldots, n\}$. Set $T_0 = 0$. The process $\{(J_k,\ T_k);\ k = 0, 1, \ldots\}$ is also Markov. It is intimately connected to Markov Renewal and semi-Markov processes (see e.g., Pyke, 1961; Çinlar, 1969). The Markov nature of the processes $\{J_k;\ k = 0, 1, \ldots\}$ and $\{(J_k,\ T_k);\ k = 0, 1, \ldots\}$ is a consequence of the underlying process $\{X(t); t \geq 0\}$ being Markov.

The various processes referred to in the previous paragraph are illustrated in Fig 1. All that can be observed experimentally is the sequence of observed sojourns T_1, T_2, ... Thus the process $\{(J_k, T_k); k = 0, 1, \ldots\}$ more than describes the observed single channel kinetics incorporating time interval omission. Its mathematical properties are completely determined by the matrix function $F(t)$ $(t > 0)$, defined elementwise by

$$F_{ij}(t) = Pr(T_k \le t \text{ and } J_k = j | J_{k-1} = i) \quad (i, j = 1, 2, \ldots, n).$$

Note that the transition matrix, P^J say, of the Markov chain $\{J_k; k = 0, 1, \ldots\}$ is given by $P^J = F(\infty)$.

We are unable to provide a closed form expression for $F(t)$. However, we can obtain its Laplace transform, $\Phi(\theta)$, from which several important properties concerning single channel records incorporating time interval omission, including moments and autocorrelation functions, can be derived.

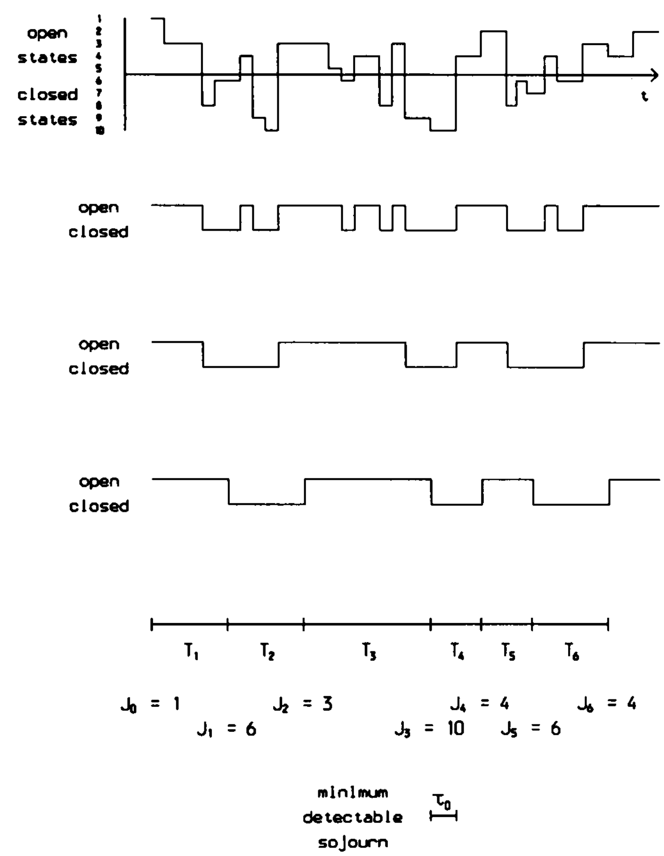


FIGURE 1 Diagram depicting the processes involved in modeling single channel data with time interval omission. The top graph shows the underlying process $\{X[t]; t \ge 0\}$. States 1 to 5 are open and 6 to 10 closed. The second graph shows the process that is in principle observable. However short sojourns remain undetected so the observed process is as shown in the third graph. The bottom graph shows how the observed process is modeled, in that sojourns become detected after a time lag τ_0; T_k is the length of the kth observed sojourn and J_k is the state the underlying process is in when the $(k+1)$st sojourn is detected.

For $\theta \ge 0$ let $\Phi(\theta)$ be the $n \times n$ matrix with elements

$$\phi_{ij}(\theta) = \int_0^\infty \exp(-\theta t)\, dF_{ij}(t) \; (i, j = 1, 2, \ldots, n). \quad (2)$$

Then it follows from Ball and Sansom (1988) that

$$\Phi(\theta) = -\{Q_0(\theta) - Q_1 Q_0(\theta)^{-1} \cdot (I - \exp[\tau_0 Q_0(\theta)])Q_1\}^{-1} Q_1 \exp[\tau_0 Q_0(\theta)] \quad (\theta \ge 0) \quad (3)$$

where

$$Q_0(\theta) = -(\theta I - Q_0),$$

$$\exp[\tau_0 Q_0(\theta)] = \sum_{k=0}^{\infty} \tau_0^k Q_0(\theta)^k / k!$$

is the usual matrix exponential (see e.g., Bellman, 1960) and, lest there be any confusion,

$$Q_0(\theta)^k = [-(\theta I - Q_0)]^k \quad (k = -1, 0, 1, \ldots).$$

Eq. 3 can also be derived by appropriate use of Eq. 16c of Roux and Sauvé (1985). Their matrix function $F(\tau, \tau_m)$ is comparable with our $dF(t)$ (with τ and τ_m broadly corresponding to t and τ_0 respectively), though they describe slightly different events. However, it must be stressed that Roux and Sauvé (1985) only considered $F(\tau, \tau_m)$ within the context of a single observed sojourn, whereas our $F(t)$ is a cornerstone of a mathematical framework describing an observed single channel record. Roux and Sauvé (1985) derived $F(\tau, \tau_m)$ by summing the probability densities of all paths of the underlying process $\{X(t); t \ge 0\}$ which yield an observed sojourn of length τ. They introduced a Dirac delta function to take account of an inequality constraint, which resulted in their obtaining a complex integral expression for $F(\tau, \tau_m)$. Their expression may be viewed as an inverse Fourier transform. Ball and Sansom (1988) derived $\Phi(\theta)$ directly, exploiting the conditional independence property of the sample paths of $\{X(t); t \ge 0\}$ and a regenerative phenomenon of sample paths of $\{X(t); t \ge 0\}$ comprising an observed sojourn. Ball and Sansom's method carries over,

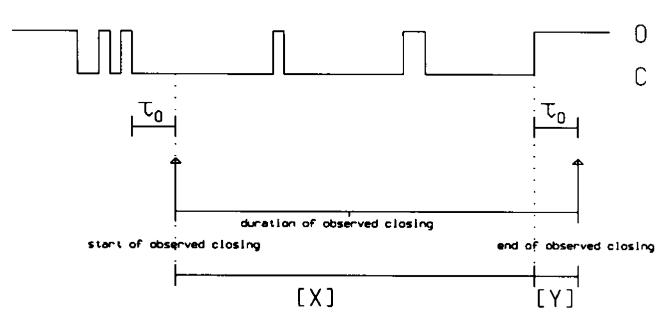


FIGURE 2 A diagram of the dynamic events comprising an observed closed sojourn, as described by Eq. 3: $\Phi(\theta) = [X][Y]$, where

$$[X] = [Q_0(\theta) - Q_1 Q_0(\theta)^{-1}\{I - \exp[\tau_0 Q_0(\theta)]\}Q_1]^{-1} Q_1$$

$$[Y] = \exp[\tau_0 Q_0(\theta)]$$

and τ_0 is the minimum detectable sojourn.

with no real modification, to the Fourier transform of $dF(t)$. The difference between the two derivations then becomes very clear; Roux and Sauvé (1985) operate in the time domain throughout, whereas Ball and Sansom (1988) operate in the frequency domain throughout.

The components comprising Eq. 3 for $\Phi(\theta)$ correspond to dynamic events as illustrated in Fig. 2.

Note that from Eq. 2,

$$\phi_{ij}(0) = \int_0^\infty dF_{ij}(t) = F_{ij}(\infty) \ (i, j = 1, 2, \ldots, n)$$

so

$$P^J = -\{Q_0 - Q_1 Q_0^{-1}[I - \exp(\tau_0 Q_0)]Q_1\}^{-1} Q_1 \exp(\tau_0 Q_0). \quad (4)$$

It will be useful for the sequel to partition P^J into

$$P^J = \begin{bmatrix} 0 & P^J_{oc} \\ P^J_{co} & 0 \end{bmatrix}.$$

Equilibrium Probabilities

In order to determine unconditional moments and autocorrelations for the observed process incorporating time interval omission we need to derive appropriate equilibrium probabilities. The Markov chain $\{J_k; k = 0, 1, \ldots\}$ will not possess an equilibrium distribution, since it has period 2, i.e., the channel alternates between closed (k odd) and open (k even) states. Consider the Markov chain $\{J_{2k}; k = 0, 1, \ldots\}$, which we term the open entry process as it records the state that the underlying process $\{X(t); t \geq 0\}$ is in whenever a channel opening is detected. The open entry process has a transition matrix, P^J_0 say, given by the top left $n_o \times n_o$ submatrix of $(P^J)^2$. It will also possess an equilibrium distribution, $\pi_o = (\pi^o_1, \pi^o_2, \ldots, \pi^o_{n_o})^T$ say, which can be obtained either from the eigenvector of P^J_0 corresponding to the eigenvalue one, or as follows.

Let $R = (r_{ij})$ be the $n \times n$ matrix given by

$$R = \exp(\tau_0 Q_0) Q_1 \{I - [Q_0^{-1}\{I - \exp(\tau_0 Q_0)\}Q_1]^2\}^{-1} \exp(\tau_0 Q_0) \quad (5)$$

and

$$\eta_i = \sum_{j=1}^{n} r_{ij}.$$

Then

$$\pi^o_i = \alpha \pi_i \eta_i \ (i \in O), \quad (6)$$

where α is a normalising constant, chosen so that the equilibrium probabilities π^o_i ($i \in O$) sum to unity. Recall that π_i is the equilibrium probability that the underlying process $\{X(t); t \geq 0\}$ is in state i.

The above expression for the equilibrium distribution π_o is proved formally, in a more general setting, in Ball and Sansom (1988). We now outline an informal proof. The equilibrium distribution π_o will be identical to the equilibrium distribution of the reversed process of the Markov chain $\{J_{2k}; k = 0, 1, \ldots\}$. The reversed process of the open entry process $\{J_{2k}; k = 0, 1, \ldots\}$ can be viewed as the open exit process of the reversed process of $\{X(t); t \geq 0\}$. However, the underlying process $\{X(t); t \geq 0\}$ is time reversible, so the equilibrium distribution π_o will be identical to the equilibrium distribution of the open exit process of $\{X(t); t \geq 0\}$. The equilibrium probability that $\{X(t); t \geq 0\}$ is in open state i is π_i, thus the equilibrium probability that the open exit process is in state i, and hence π^o_i, is directly proportional to the product of π_i and the rate (or probability density) of exiting from state i. An exiting path from open state i will consist of an initial sojourn of length τ_0 in the open states, whence the underlying process $\{X(t); t \geq 0\}$ enters the closed states and thereafter the first sojourn of $\{X(t); t \geq 0\}$ of length at least τ_0 is in the closed states. (If the first such sojourn was in the open states then state i in the above would not be an exit state.) Let j be the closed state that the underlying process $\{X(t); t \geq 0\}$ is in when entry into the closed states is detected. For $i \in O$ and $j \in C$ let r_{ij} be the rate of exiting from open state i to closed state j. Similarly define r_{ij} for $i \in C$ and $j \in O$, set $r_{ij} = 0$ for $i, j \in O$ and $i, j \in C$ and let R be the $n \times n$ matrix with elements r_{ij}. Then the rate of exiting from state i is $\Sigma^n_{j=1} r_{ij}$ and all that is required to complete the proof of Eq. 6 is to show that R is given by Eq. 5. For $i \in O$ and $j \in C$, r_{ij} may be found by summing the probability density of all paths that exit from open state i to closed state j. It is simpler to partition R into

$$R = \begin{bmatrix} 0 & R_{oc} \\ R_{co} & 0 \end{bmatrix}$$

and compute R_{oc} and R_{co} directly. The details are given in Ball and Sansom (1988). For an exit from the open to the closed states, the components comprising the formula (5) for R correspond to dynamic events as follows. The term $\exp(\tau_0 Q_0) Q_1$ corresponds to the initial sojourn of length τ_0 in the open states followed by transition into the closed states. The final term $\exp(\tau_0 Q_0)$ corresponds to the final detected sojourn of length τ_0 in the closed states. The middle term $\{I - [Q_0^{-1}\{I - \exp(\tau_0 Q_0)\}Q_1]^2\}^{-1}$ corresponds to the possible sequence of pairs of undetected sojourns in the closed and open states. A similar decomposition holds for an exit from the closed to the open states.

When there is no time interval omission (i.e., $\tau_0 = 0$) the above informal proof is similar to one given in Colquhoun and Hawkes (1977).

Similarly, the equilibrium probabilities π^c_i ($i \in C$) of the closed entry process $(J_{2k+1}; k = 0, 1, \ldots)$ are given by

$$\pi^c_i = \beta \pi_i \eta_i \quad (i \in C),$$

where β is another normalising constant. Alternatively, the equilibrium distribution of the closed entry process can be derived using the equation

$$\pi_c = (P^J_{oc})^\tau \pi_o.$$

Note that when there is no time interval omission, i.e., $\tau_0 =$

0, the above formula for entry process equilibrium probabilities reduce to equivalent results given in Colquhoun and Hawkes (1977).

Moments

It is important to be able to evaluate the effect of time interval omission on the observed mean sojourn times, particularly if they are to be used in evaluating single channel dose-response curves (see e.g., Kerry et al., 1987*b*). Furthermore, first and second moments are required for calculation of autocorrelation functions. The moments of observed sojourns in the open and closed states can be obtained from the Laplace transform $\Phi(\theta)$ in the usual way (e.g., Mood et al., 1974). Let $M^{(r)}$ $(r = 1, 2, \ldots)$ be the $n \times n$ matrix with elements

$$\mu_{ij}^{(r)} = \int_0^\infty t^r \, dF_{ij}(t) \quad (i, j = 1, 2, \ldots, n).$$

Then

$$M^{(r)} = (-1)^{(r)}\Phi^{(r)}(0) \quad (r = 1, 2, \ldots)$$

where $\Phi^{(r)}(\theta)$ is the matrix function obtained by differentiating $\Phi(\theta)$ elementwise r times with respect to θ. For $r = 1$ and 2 we obtain

$$M^{(1)} = U^{-1}\{(Q_0^{-2} - \tau_0 Q_0^{-1})Q_1 \exp(\tau_0 Q_0) - VQ_1 P^J\}$$

and

$$M^{(2)} = U^{-1}\{-(2Q_0^{-3} - 2\tau_0 Q_0^{-2} + \tau_0^2 Q_0^{-1})Q_1 \cdot \exp(\tau_0 Q_0) - 2VQ_1 M^{(1)} + [2Q^{(3)} - \tau_0^2 Q^{(1)} - 2(Q^{(3)} - \tau_0 Q^{(2)}) \exp(\tau_0 Q_0)]Q_1 P^J\},$$

where

$$Q^{(1)} = Q_0^{-1} Q_1 Q_0^{-1}$$

$$Q^{(2)} = Q_0^{-2} Q_1 Q_0^{-1} + Q_0^{-1} Q_1 Q_0^{-2}$$

$$Q^{(3)} = Q_0^{-3} Q_1 Q_0^{-1} + Q_0^{-2} Q_1 Q_0^{-2} + Q_0^{-1} Q_1 Q_0^{-3}$$

$$U = I - Q^{(1)}[I - \exp(\tau_0 Q_0)]Q_1$$

and

$$V = (\tau_0 Q^{(1)} - Q^{(2)}) \exp(\tau_0 Q_0) + Q^{(2)}.$$

For $i \in O$ let $\mu_i^{(r)}$ be the *rth* moment of observed sojourns in the open state with entry state i. Similarly, define $\mu_i^{(r)}$ for $i \in C$, and let $\mu^{(r)} = (\mu_1^{(r)}, \mu_2^{(r)}, \ldots, \mu_n^{(r)})^T$. Then by summing over possible entry states for the succeeding sojourn we have

$$\mu^{(r)} = M^{(r)}\mathbf{1} \quad (r = 1, 2, \ldots), \tag{7}$$

where $\mathbf{1}$ is the $n \times 1$ column vector of ones.

Finally, let $\mu_o^{(r)}$ and $\mu_c^{(r)}$ be the unconditional *rth* moments of observed sojourns in the open and closed states, respectively. Then

$$\mu_o^{(r)} = \sum_{i=1}^{n_o} \pi_i^o \mu_i^{(r)} \tag{8}$$

and

$$\mu_c^{(r)} = \sum_{i=n_o+1}^{n} \pi_i^c \mu_i^{(r)}.$$

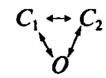
Covariance Functions

Let m_o be the number of open gateway states, i.e., open states from which direct transition into the closed states is possible, m_c the number of closed gateway states, and $m = \min(m_o, m_c)$. For example:

I

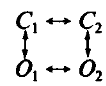
$$C_1 \leftrightarrow O \leftrightarrow C_2 \quad m = 1$$

II



$m = 1$

III



$m = 2$

Let $S_1, S_2, \ldots$ be the lengths of successive sojourns in the open states. Thus, in our earlier notation, $S_k = T_{2k-1}$ $(k = 1, 2, \ldots)$. Fredkin et al. (1985) showed that when there is no time interval omission $(\tau_0 = 0)$ and the process is in equilibrium.

$$\mathrm{Cov}(S_i, S_{i+k}) = \sum_{j=1}^{m-1} v_j \sigma_j^{|k|} \quad (k \neq 0), \tag{9}$$

where $0 \le \sigma_j < 1$ $(j = 1, 2, \ldots, m - 1)$, with a similar result holding for the closed sojourns. The σ_j's in Eq. 9 are the eigenvalues, that are not zero or one, of the transition matrix, P_o^J, of the open entry process when time interval omission is absent $(\tau_0 = 0)$. Fredkin et al. (1985) showed that there are at most $m - 1$ such eigenvalues and indicate that there will usually be exactly $m - 1$ such eigenvalues. For $j = 1, 2, \ldots, m - 1$, v_j of Eq. 9 is associated with the component of the spectral decomposition of P_o^J corresponding to the eigenvalue σ_j. Expressions for v_j $(j = 1, 2, \ldots, m - 1)$ are given in Fredkin et al. (1985). The values of v_j $(j = 1, 2, \ldots, m - 1)$ may also be obtained by implicitly setting $\tau_0 = 0$ in the formula after Eq. 15 below.

When $m = 1$, as in mechanisms I and II above, all the covariances are zero. Thus by fitting functions of the form of Eq. 9 to autocovariance functions estimated from experimental records an estimate of the degree of connectivity of the open and closed states can, at least in principle, be obtained (Fredkin et al., 1985). However, in deriving Eq. 9, it is assumed that $\tau_0 = 0$. It is of crucial importance to understand the effect of time interval omission on the form of the autocovariance functions if the latter are to be reliably interpreted in terms of underlying channel gating mechanisms. We now provide expressions for the autocovariance functions when time interval omission is incorporated. Their interpretation will be discussed later in the paper, and a particular example will be studied in some detail.

Let $M = M^{(1)}$ be the matrix pertaining to mean observed sojourns and partition M into

$$M = \begin{bmatrix} 0 & M_o \\ M_c & 0 \end{bmatrix}.$$

Then, provided the observed process is in equilibrium, Ball and Sansom (1988) show that

$$\text{Cov}\,(S_i, S_{i+k}) = \pi_o^T M_o P_{\infty}^J (P_o^J)^{|k|-1} M_o \mathbf{1} - (\mu_o^{(1)})^2 \quad (k \neq 0). \quad (10)$$

Similarly, if we let $S_1', S_2', \ldots$ be the lengths of successive observed closed sojourns, so that $S_k' = T_{2k}$ $(k = 1, 2, \ldots)$ in our earlier notation, then

$$\text{Cov}\,(S_i', S_{i+k}') = \pi_c^T M_c P_{\infty}^J (P_c^J)^{|k|-1} M_c \mathbf{1} - (\mu_c^{(1)})^2 \quad (k \neq 0). \quad (11)$$

Now suppose that the transition matrix P_o^J of the open entry process is diagonalisable. Let $\lambda_1, \lambda_2, \ldots, \lambda_{n_o}$ by the eigenvalues of P_o^J, with corresponding right eigenvectors $\mathbf{b}_1, \mathbf{b}_2, \ldots, \mathbf{b}_{n_o}$. Let B be the $n_o \times n_o$ matrix, whose ith column is $\mathbf{b}_i$, and $C = B^{-1}$. It follows that P_o^J admits the spectral representation

$$P_o^J = \sum_{i=1}^{n_o} \lambda_i E_i, \quad (12)$$

where

$$E_i = \mathbf{b}_i \mathbf{c}_i,$$

$\mathbf{c}_i$ being the ith row of c.

The matrices $E_1, E_2, \ldots, E_{n_o}$ satisfy

$$E_i E_j = 0 \quad (i \neq j),$$

$$E_i^2 = E_i,$$

and

$$E_1 + E_2 + \ldots + E_{n_o} = I.$$

Furthermore, one of the eigenvalues, λ_1 say, is unity and the remainder have modulus strictly less than one (Cox and Miller, 1965). Also, all the rows of E_1 will be π_o^T. Moreover, it can be shown (Ball and Sansom, 1988) that P_o^J has rank at most m, so at most m of the λ_i's will be nonzero. Thus Eq. 12 may be written

$$P_o^J = E_1 + \sum_{i=2}^{m} \lambda_i E_i, \quad (13)$$

where the summation is omitted if $m = 1$.

Substituting Eq. 13 into Eq. 10 yields

$$\text{Cov}\,(S_i, S_{i+k}) = \pi_o^T M_o P_{\infty}^J \left(\sum_{j=1}^{m} \lambda_j^{|k|-1} E_j \right) \cdot M_o \mathbf{1} - (\mu_o^{(1)})^2 \quad (k \neq 0). \quad (14)$$

As noted above, $E_1 = \mathbf{1}\pi_o^T$. Hence, $P_{\infty}^J E_1 = \mathbf{1}\pi_o^T$, since the rows of P_{∞}^J each sum to unity. Thus the term corresponding to E_1 in Eq. 14 reduces to $(\pi_o^T M_o \mathbf{1})^2$, which equals $(\mu_o^{(1)})^2$, using Eqs. 7 and 8. Thus

$$\text{Cov}\,(S_i, S_{i+k}) = \pi_o^T M_o P_{\infty}^J \left(\sum_{j=2}^{m} \lambda_j^{|k|-1} E_j \right) M_o \mathbf{1}, \quad (15)$$

which may be expressed in the form of Eq. 9, with $\sigma_j = \lambda_{j+1}$ and $v_j = \pi_o^T M_o P_{\infty}^J E_{j+1} M_o \mathbf{1}$ $(j = 1, 2, \ldots, m-1)$.

Let $\Gamma_o(k) = \text{corr}(S_i, S_{i+k})$ be the autocorrelation function for successive observations in the open states and similarly define $\Gamma_c(k)$ for closed sojourns. Then $\Gamma_o(k) = \text{Cov}\,(S_i, S_{i+k})/\text{Var}\,(S_i)$ and it follows from Eq. 15 that $\Gamma_o(k)$ may be expressed in the form

$$\Gamma_o(k) = \sum_{j=1}^{m-1} \nu_j \sigma_j^{|k|} \quad (k \neq 0), \quad (16)$$

where $\sigma_j = \lambda_{j+1}$ and $\nu_j = \pi_o^T M_o P_{\infty}^J E_{j+1} M_o \mathbf{1} / (\text{Var}\,(S_i)\lambda_{j+1})$ $(j = 1, 2, \ldots, m-1)$. Thus $|\sigma_j| < 1$ $(j = 1, 2, \ldots, m-1)$.

Similarly, if the transition matrix P_c^J of the closed entry process is diagonalisable, with eigenvalues $\mu_1 = 1, \mu_2, \ldots, \mu_{n_c}$, of which at most m are nonzero, and spectral representation

$$P_c^J = F_1 + \sum_{i=2}^{m} \mu_i F_i, \quad (17)$$

then

$$\text{Cov}\,(S_i', S_{i+k}') = \pi_c^T M_c P_{\infty}^J \left(\sum_{j=2}^{m} \mu_j^{|k|-1} F_j \right) M_c \mathbf{1} \quad (k \neq 0), \quad (18)$$

and thus $\Gamma_c(k) = \text{Cov}\,(S_i', S_{i+k}')/\text{Var}\,(S_i')$ may be expressed in the form

$$\Gamma_c(k) = \sum_{j=1}^{m-1} \upsilon_j \kappa_j^{|k|} \quad (k \neq 0), \quad (19)$$

where $\kappa_j = \mu_{j+1}$ and $\upsilon_j = \pi_c^T M_c P_{\infty}^J F_{j+1} M_c \mathbf{1} / (\text{Var}(S_i')\lambda_{j+1})$ $(j = 1, 2, \ldots, m-1)$. Thus $|\kappa_j| < 1$ $(j = 1, 2, \ldots, m-1)$.

Note that representations (16) and (19) for the autocorrelation functions $\Gamma_o(k)$ and $\Gamma_c(k)$ have assumed that the transition matrices P_o^J and P_c^J are diagonalisable. When there is no time interval omission, i.e. $\tau_0 = 0$, P_o^J and P_c^J are necessarily diagonalisable, with real positive eigenvalues (Fredkin et al., 1985). When time interval omission is incorporated it no longer seems straightforward to show that P_o^J and P_c^J are diagonalisable with positive real eigenvalues. However, in all of our numerical studies these matrices have been diagonalisable with positive real eigenvalues, and we strongly conjecture that this will generally be the case.

COMPUTATIONAL CONSIDERATIONS

Numerical calculation of the formula of the previous section is straightforward to implement on a computer. We now outline the salient points in performing such a numerical study. Our starting point is the infinitesimal transition matrix Q of the underlying process $\{X(t); t \geq 0\}$. Firstly, we calculate the equilibrium distribution π of the underlying process $\{X(t); t \geq 0\}$, using the detailed balance conditions (1) as follows. Set $\pi_1 = 1$, calculate π_i $(i = 2, 3, \ldots, n)$ from Eq. 1 and then normalize the resulting π_i's so that they sum to unity. Now form the $n \times n$ diagonal matrix W with elements $w_{ii} = \pi_i$ $(i = 1, 2, \ldots, n)$. It follows from the detailed balance conditions (1) that the matrices

$$\tilde{Q}_o = W^{1/2} Q_o W^{-1/2} \tag{20}$$

and

$$\tilde{Q}_1 = W^{1/2} Q_1 W^{-1/2} \tag{21}$$

are symmetric and hence diagonalisable (see Fredkin et al., 1985).

Note that

$$\begin{aligned}\exp(\tau_0 \tilde{Q}_0) &= \sum_{k=0}^{\infty} \tau_0^k \tilde{Q}_0^k / k! \\ &= W^{1/2} \left(\sum_{k=0}^{\infty} \tau_0^k Q_0^k / k! \right) W^{-1/2} \\ &= W^{1/2} \exp(\tau_0 Q_0) W^{-1/2},\end{aligned}$$

and

$$\exp(\tau_0 Q_0) = W^{-1/2} \exp(\tau_0 \tilde{Q}_0) W^{1/2}. \tag{22}$$

Further, using Eqs. 20 and 21, it is straightforward to show that many of the formula of the previous section can be expressed in a similar form to Eq. 22; e.g.,

$$P^J = -W^{-1/2}\{\tilde{Q}_0 - \tilde{Q}_1 \tilde{Q}_0^{-1} \cdot [I - \exp(\tau_0 \tilde{Q}_0)] \tilde{Q}_1\}^{-1} Q_1 \exp(\tau_0 \tilde{Q}_0) W^{1/2}.$$

The advantage of doing calculations in terms of $\tilde{Q}_0$ and $\tilde{Q}_1$ is that many of the matrices encountered are symmetric, permitting accurate and efficient calculations of eigenvalues, eigenvectors and inverses.

There are many ways of calculating $\exp(\tau_0 \tilde{Q}_0)$, (Moler and Van Loan, 1978). The simplest for our purposes is to use the spectral representation of $\tilde{Q}_0$. Specifically let $\gamma_1, \gamma_2, \ldots, \gamma_n$ be the (strictly negative real) eigenvalues of $\tilde{Q}_0$ with a corresponding set of orthonormal right eigenvectors $\mathbf{x}_1, \mathbf{x}_2, \ldots, \mathbf{x}_n$. Let $H_1, H_2, \ldots, H_n$ be $n \times n$ matrices defined by

$$H_i = \mathbf{x}_i \mathbf{x}_i^T \ (i = 1, 2, \ldots, n).$$

Then

$$\tilde{Q}_0 = \sum_{i=1}^{n} \gamma_i H_i$$

and the matrices $H_1, H_2, \ldots, H_n$ satisfy

$$H_i H_j = \begin{cases} H_i & \text{if } i = j \\ \mathbf{0} & \text{if } i \neq j, \end{cases}$$

and

$$H_1 + H_2 + \ldots + H_n = I.$$

It then follows from the definition of $\exp(\tau_0 \tilde{Q}_0)$ that

$$\exp(\tau_0 \tilde{Q}_0) = \sum_{i=1}^{n} \exp(\gamma_i \tau_0) H_i.$$

All the formula of the previous section are now straightforward to compute. The autocovariance functions can be computed directly from Eqs. 10 and 11. However, it is also worthwhile diagonalising the matrices P_o^J and P_c^J, and using Eqs. 13 and 17 to express the autocorrelation functions in the forms Eqs. 16 and 19, thus allowing comparison with the case of no time interval omission, $\tau_0 = 0$. In doing this one should bear in mind that currently there is no theoretical proof that P_o^J and P_c^J are diagonalisable, nor that their eigenvalues and eigenvectors are real.

APPLICATIONS

Interpreting Autocorrelation Functions

As we have already noted, when no time interval omission is present a lower bound for $m = \min(m_o, m_c)$ can, in principle, be obtained by fitting expressions of the form of Eqs. 16 and 19 to estimated autocorrelation functions for observed open and closed sojourns. A lower bound on m is an important constraint when considering possible gating mechanisms to explain observed channel kinetics. A natural question to ask is can a similar inference be made when time interval omission is present? Clearly, if not then analysis of observed autocorrelation functions would be of limited value. We have shown that, provided the transition matrices P_o^J and P_c^J are diagonalisable with positive real eigenvalues, then expressions of the form of Eqs. 16 and 19 still hold when time interval omission is incorporated, though the correlations themselves may be different, and thus inferences of the above type can still be made. So, even in the presence of time interval omission, a lower bound on m may be obtained.

The problem of fitting expressions of the form (16) to estimated autocorrelation functions is briefly considered later.

Time Interval Omission and Zero Correlations

A second question of considerable importance is whether time interval omission may give rise to nonzero correlations which otherwise would not be present. For example, the mechanism shown below

$$\underset{1}{\mathrm{O}} \rightleftharpoons \underset{2}{\mathrm{O}} \rightleftharpoons \underset{3}{\mathrm{C}} \rightleftharpoons \underset{4}{\mathrm{C}} \rightleftharpoons \underset{5}{\mathrm{C}}$$

has $m = 1$, so when there is no time interval omission successive open sojourns will have zero correlation, indeed they are independent. Will this still be the case when time interval omission is introduced, i.e., $\tau_0 > 0$? Firstly note that since rank $(P_o^J) \le m$, we must have rank $(P_o^J) = 1$. We show in the appendix that P_o^J also admits the spectral representation (13), with $m = 1$, so it follows that successive observed open sojourns are uncorrelated. Alternatively we may argue as follows. For the above linear scheme each detected open sojourn must have commenced with a transition from state 3 to state 2 so, since the underlying process $\{X(t); t \ge 0\}$ is Markov, successive observed open sojourns are independent and hence uncorrelated. Similar arguments hold for closed sojourns.

We have demonstrated this using simulations for the above mechanism. The results (Fig. 3) clearly indicate that no autocorrelation is seen, either in the presence ($\tau_0 = 0.2$ ms) or absence ($\tau_0 = 0.0$ *ms*) of time interval omission.

More generally, in the absence of time interval omission, zero correlations for open sojourns will occur if either P_o^J has rank 1 or the parameters of the underlying process are such that

$$\sum_{j=1}^{m-1} \nu_j \sigma_j^{|k|} = 0 \text{ for all } k \neq 0.$$

We concentrate here on the former case, indeed it is far from clear that the latter is possible unless P_o^J has rank 1. Write P_o^J and P_c^J as $P_o^J(\tau_0)$ and $P_c^J(\tau_0)$ to show their dependence on τ_0. We show in the appendix that $P_o^J(0)$ has rank 1 if and only if $P_c^J(0)$ has rank 1. Moreover, we show further that either of these conditions imply that, for all $\tau_0 > 0$, the matrices $P_o^J(\tau_0)$ and $P_c^J(\tau_0)$ both have rank 1 and admit appropriate spectral representations. It follows that both observed open and closed sojourns will still have zero correlations. Thus in this quite general setting the presence of time interval omission will not give rise to spurious correlations, provided of course that the underlying Markov model is correct.

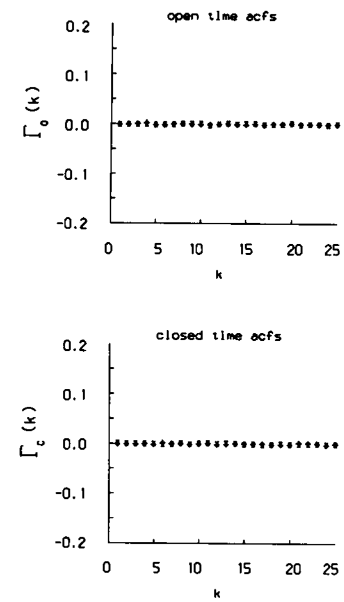


FIGURE 3 Sample autocorrelation functions for the linear gating mechanism:

$$\underset{1}{O} \rightleftharpoons \underset{2}{O} \rightleftharpoons \underset{3}{C} \rightleftharpoons \underset{4}{C} \rightleftharpoons \underset{5}{C}$$

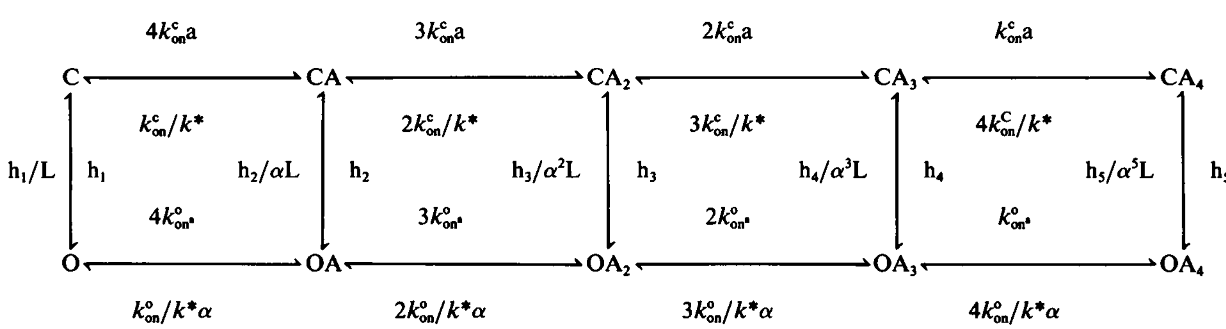
for which the transition matrix was

$$Q = \begin{pmatrix} -0.1 & 0.1 & 0 & 0 & 0 \\ 10 & -20 & 10 & 0 & 0 \\ 0 & 1{,}000 & -2{,}000 & 1{,}000 & 0 \\ 0 & 0 & 1{,}000 & -2{,}000 & 1{,}000 \\ 0 & 0 & 0 & 1{,}000 & -1{,}000 \end{pmatrix},$$

where the units are s^{-1}. The autocorrelations are derived from simulations of 10^6 channel openings, both without ($\tau_0 = 0.0$ *ms*; +) and with ($\tau_0 = 0.2$ *ms*; ×) time interval omission. In both cases, a null autocorrelation function is seen.

An Allosteric Model for Channel Gating

Several studies (Karlin, 1967; Colquhoun and Hawkes, 1977) have suggested the use of allosteric models for channel gating mechanisms. The following mechanism, with four agonist binding sites, has proved useful in understanding the gating kinetics of the locust muscle glutamate receptor-channel (Kerry et al., 1987*a*, *b*):-



where A_n represents n molecules of agonist bound to the receptor channel, C the channel being closed, and O the channel being open. The transition rates for the underlying continuous time Markov chain are also shown, where a is the agonist concentration.

Current estimates of the parameters of the above model, together with their appropriate units, are as follows:

$$k_{on}^{c} = 10\,ms^{-1}\,M^{-1} \quad k_{on}^{o} = 1\,ms^{-1}\,M^{-1} \quad k^* = 2{,}000\,M^{-1}$$

$$L = 6 \times 10^{-4} \quad \alpha = 10 \quad h_1 = 10^{-2}\,ms^{-1}$$

$$h_2 = 1.5 \times 10^{-2}\,ms^{-1} \quad h_2 = 2.6 \times 10^{-2}\,ms^{-1}$$

$$h_4 = 6.7 \times 10^{-1}\,ms^{-1} \quad h_5 = 1.7 \times 10^{-1}\,ms^{-1}.$$

These estimates are based on those in Kerry et al. (1987*b*) and form a working hypothesis which explains some of the observed kinetics of the glutamate receptor-channel. More systematic methods of parameter estimation are currently under investigation.

Details of Calculations for $a = 10^{-4}\,M$ and $\tau_0 = 0.2$ ms

We will examine in some detail the situation where $a = 10^{-4}\,M$ and $\tau_0 = 0.2\,ms$. This corresponds to the experimental work of Kerry et al. (1987*a*).

We label 1, 2, 3, 4, 5 the open states O, OA, OA_2, OA_3, OA_4, and 6, 7, 8, 9, 10 the closed states C, CA, CA_2, CA_3, CA_4. For the above cyclic model the equilibrium distribution π is given by

$$\pi_i = \begin{cases} \binom{4}{i-1}(ak^*\alpha)^{i-1}c & i = 1, 2, \ldots, 5 \\ \binom{4}{i-6}(ak^*)^{i-6}c/L & i = 6, 7, \ldots, 10 \end{cases}$$

where $c = [(1 + ak^*\alpha)^4 + (1 + ak^*)^4/L]^{-1}$ (Ball and Sansom, 1987). For the above parameter values we find

$$\pi_1 = 0.0003 \quad \pi_2 = 0.0023 \quad \pi_3 = 0.0068 \quad \pi_4 = 0.0090$$

$$\pi_5 = 0.0045 \quad \pi_6 = 0.4712 \quad \pi_7 = 0.3770$$

$$\pi_8 = 0.1131 \quad \pi_9 = 0.0151 \quad \pi_{10} = 0.0008.$$

Note that the channel is predominantly closed with few, if any, molecules of agonist bound to it. The equilibrium probability of the channel being open is therefore 0.0229.

The transition matrix of the embedded Markov chain $\{J_n;\, n = 0, 1, \ldots\}$ is

$$P^J = \begin{bmatrix} 0.0000 & 0.0000 & 0.0000 & 0.0000 & 0.0000 & 0.9992 & 0.0008 & 0.0000 & 0.0000 & 0.0000 \\ 0.0000 & 0.0000 & 0.0000 & 0.0000 & 0.0000 & 0.0010 & 0.9983 & 0.0007 & 0.0000 & 0.0000 \\ 0.0000 & 0.0000 & 0.0000 & 0.0000 & 0.0000 & 0.0000 & 0.0022 & 0.9969 & 0.0009 & 0.0000 \\ 0.0000 & 0.0000 & 0.0000 & 0.0000 & 0.0000 & 0.0000 & 0.0000 & 0.0034 & 0.9963 & 0.0003 \\ 0.0000 & 0.0000 & 0.0000 & 0.0000 & 0.0000 & 0.0000 & 0.0000 & 0.0000 & 0.0112 & 0.9887 \\ 0.1141 & 0.7158 & 0.1573 & 0.0128 & 0.0000 & 0.0000 & 0.0000 & 0.0000 & 0.0000 & 0.0000 \\ 0.0351 & 0.7796 & 0.1713 & 0.0140 & 0.0000 & 0.0000 & 0.0000 & 0.0000 & 0.0000 & 0.0000 \\ 0.0098 & 0.2179 & 0.7140 & 0.0582 & 0.0001 & 0.0000 & 0.0000 & 0.0000 & 0.0000 & 0.0000 \\ 0.0003 & 0.0059 & 0.0194 & 0.9727 & 0.0016 & 0.0000 & 0.0000 & 0.0000 & 0.0000 & 0.0000 \\ 0.0000 & 0.0006 & 0.0021 & 0.1029 & 0.8943 & 0.0000 & 0.0000 & 0.0000 & 0.0000 & 0.0000 \end{bmatrix},$$

from which the transition matrix of the open entry process is found to be

$$P_o^J = \begin{bmatrix} 0.1140 & 0.7158 & 0.1573 & 0.0128 & 0.0000 \\ 0.0352 & 0.7791 & 0.1717 & 0.0140 & 0.0000 \\ 0.0099 & 0.2189 & 0.7122 & 0.0589 & 0.0001 \\ 0.0003 & 0.0066 & 0.0218 & 0.9694 & 0.0019 \\ 0.0000 & 0.0007 & 0.0023 & 0.1127 & 0.8843 \end{bmatrix}.$$

The diagonal elements of P_o^J are generally quite large, except for the first one, which corresponds to no molecules of agonist being bound to the channel. An explanation for this small value can be obtained from the matrix P^J. The probability of the entry process going from state 1 to state 6 is very close to one, however that for going from state 6 to state 1 is quite small. This is because an entry state is by definition the state that the underlying process is in when a sojourn is detected and the underlying process has a high transition rate out of state 1, as suggested by the low value of π_1. When there is no time interval omission the diagonal elements of P_o^J are 0.7634, 0.7217, 0.7106, 0.9778, and 0.8886. The element corresponding to state 1 is no longer small since when there is no time interval omission an entry state is the state the corresponding sojourn commenced in.

The equilibrium distribution of the open entry process is

$$\pi_1^o = 0.0123(0.2002)$$

$$\pi_2^o = 0.2508(0.2402)$$

$$\pi_3^o = 0.1966(0.1249)$$

$$\pi_4^o = 0.5312(0.4292)$$

$$\pi_5^o = 0.0090(0.0054),$$

where the figures in brackets are the corresponding values when there is no time interval omission ($\tau_0 = 0$). Note that the principle difference between the two distributions is in the value of π_1^o, an explanation for which follows similar lines to that given above.

The matrix M_o pertaining to mean observed open sojourns is

$$M_o = \begin{bmatrix} 0.2601 & 0.0002 & 0.0000 & 0.0000 & 0.0000 \\ 0.0006 & 0.6004 & 0.0007 & 0.0000 & 0.0000 \\ 0.0000 & 0.0057 & 2.5108 & 0.0027 & 0.0000 \\ 0.0000 & 0.0000 & 0.0045 & 1.2329 & 0.0042 \\ 0.0000 & 0.0000 & 0.0012 & 0.4178 & 36.0399 \end{bmatrix}$$

Summing the rows of M_o, we find that the mean observed open sojourns, given the open entry state, in units of *ms*, are

$$\mu_1^{(1)} = 0.2603 \qquad \mu_2^{(1)} = 0.6017 \qquad \mu_3^{(1)} = 2.5192$$
$$\mu_4^{(1)} = 1.2416 \qquad \mu_5^{(1)} = 36.4589.$$

Taking expectations of the above values with respect to the open entry equilibrium distribution π^o, we find that the unconditional mean observed open sojourn is 1.6385 *ms*. The corresponding variance is found by similar arguments to be 25.1827 ms^2. The corresponding figures in the absence of time interval omission are 0.9729 *ms* and 14.6259 ms^2.

The transition matrices P_o^J and P_c^J of the open and closed entry processes are diagonalisable with positive real eigenvalues. For cyclic models P_o^J and P_c^J necessarily have the same set of eigenvalues, indeed we conjecture that this will be generally true. As they are also both diagonalisable it follows that $\sigma_i = \kappa_i$ ($i = 1, 2, \ldots, 5$) in Eqs. 16 and 19, and thus the autocorrelation functions $\Gamma_o(k)$ and $\Gamma_c(k)$ depend on the same set of geometrically decaying components. The parameters of $\Gamma_o(k)$ and $\Gamma_c(k)$, when given by Eqs. 16 and 19, are shown below.

i	$\sigma_i(\kappa_i)$	ν_i	υ_i
1	0.9413	0.0107	0.1737
2	0.8816	0.4416	0.0013
3	0.5581	0.0172	0.0613
4	0.0781	0.0000	0.1904

Comparison Between Calculations and Simulations

The results of the calculations discussed in the preceding section are presented alongside the results of simulation studies in Fig. 4. The autocorrelations are derived from simulated datasets of 3×10^6 observed channel openings. The agreement between the two approaches can be seen to be very good. This holds for a variety of τ_0 and a values (see below).

We have used the simulated data to tentatively explore the problem of fitting sums of geometrically decaying functions to observed autocorrelation functions. This is intended to mirror the approach adopted to the experimental data by Kerry et al. (1987*a*, *b*). The autocorrelation functions for the simulated data were fitted for k values of 1 to 25, using a standard optimization procedure. The closed time autocorrelation function is adequately described by two components, with parameters $\nu_1 = 0.176$, $\kappa_1 = 0.941$, $\nu_2 = 0.114$, and $\kappa_2 = 0.414$. The open time autocorrelation function is described by a single component, $\nu_1 = 0.450$, and $\sigma_1 = 0.881$. Comparison with the results of the previous section reveals that these estimates correspond quite closely with the dominant terms in the calculated autocorrelation functions. It would therefore seem that it is reasonable to fit such functions to experimentally derived autocorrelations in an attempt to arrive at a lower bound for m. For example, on the basis of the case just discussed, one would conclude that $m \geq 3$.

Effect of Varying the Minimum Detectable Sojourn and the Agonist Concentration

Autocorrelation functions for different values of τ_0, with $a = 10^{-4}$ *M*, are shown in Fig. 5. In this particular case there is little effect of varying τ_0 on the open time autocorrelation function. The effect on the closed time autocorrelation function is more pronounced. The overall effect of increasing the minimum detectable sojourn is to decrease the autocorrelation. Calculations over a range of a values suggest that this is generally the case. This

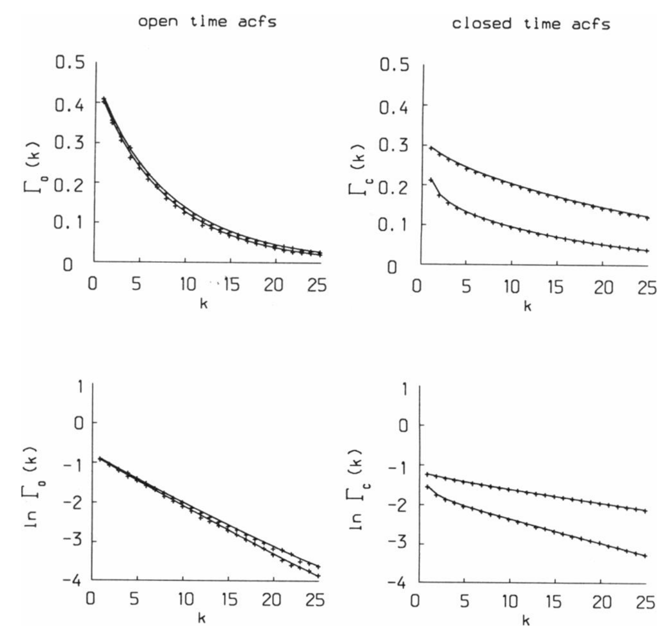


FIGURE 4 Comparison of the calculated autocorrelation functions (*solid lines*) with those derived from simulated data (*points*). The simulations were carried out for the allosteric gating mechanism described in the text, employing an agonist concentration $a = 10^{-4}M$. Each simulation was of 3×10^6 observed channel openings. In each graph the upper line corresponds to $\tau_0 = 0.0$ *ms* (i.e., no time interval omission) and the lower line to $\tau_0 = 0.2$ *ms*. The lower two graphs are constructed using log-linear scales.

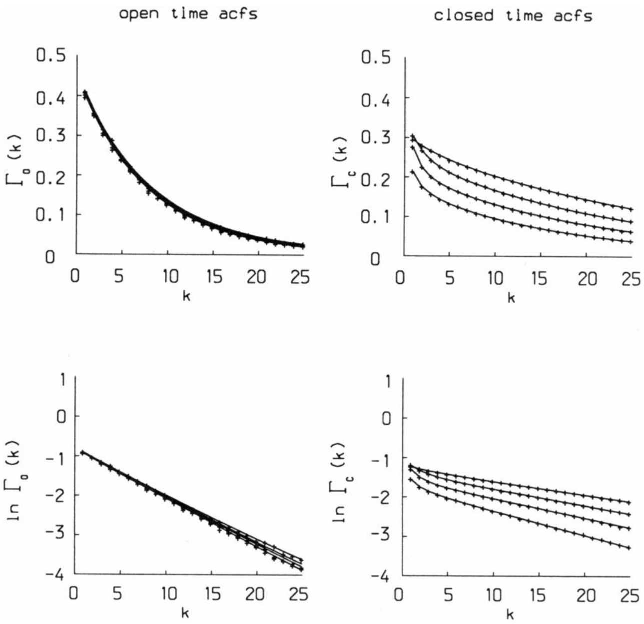


FIGURE 5 Autocorrelation functions for increasing values of τ_0. The graphs are comparable with those in Fig. 4. In each case the four lines (in descending order) correspond to $\tau_0 = 0.0, 0.05, 0.10$, and $0.20\ ms$. The simulations were carried out as described for Fig. 4.

reinforces our earlier statement that time interval omission does not generate spurious correlations.

We have also examined the behavior of the autocorrelation functions for the model presented above over a range of agonist concentrations. Positive correlations are seen for all four values of $a - 10^{-5}\,M$, $10^{-4}\,M$, $10^{-3}\,M$, and $10^{-2}\,M$ – with the same general effect of increasing the minimum detectable sojour as was discussed above. Using an upper value of k of 25, and a value of $\tau_0 = 0.2\ ms$, the simulated autocorrelation functions have been fitted with sums of geometrically decaying functions, again to obtain "experimental" estimates of the lower bound of m. As one might expect, different lower bounds to m are obtained for different agonist concentrations: $a - 10^{-5}\,M$; $m - 4$; $a = 10^{-4}\,M$; $m = 3$; $a = 10^{-3}\,M$; $m = 4$; and $a = 10^{-2}\,M$, $m = 2$. Thus one can see that, for such a mechanism, it is worthwhile to explore the channel kinetics over as wide a range of agonist concentrations as possible in order to obtain a good estimate of the lower bound on m. It is encouraging that a lower bound of 4 is obtained for an $m = 5$ model, given that correlations were only fitted out to $k = 25$.

DISCUSSION

The main objectives of the paper were twofold. First, to describe a method for calculating theoretical observed sojourn time autocorrelation functions in the presence of time interval omission. Second, to determine whether or not the presence of time interval omission invalidates inferences, concerning the degree of connectivity of the channel gating mechanism, made from the form of such autocorrelation functions.

Considering the first of the above two objectives, the method that we have described provides a general mathematical framework, that is amenable to numerical implementation, for analyzing both the dynamic and equilibrium aspects of single channel kinetics incorporating time interval omission. For example, Ball and Sansom (1987) used this framework to examine the effect of time interval omission on the temporal clustering of observed channel openings. Roux and Sauvé (1985) have provided a method of determining the pdf of observed open (closed) sojourn times, incorporating time interval omission. Before discussing the connection between their results and ours, it is convenient to consider more fully the form of time interval omission that we have used.

We have assumed that transfer into say the open states is detected after there has been a sojourn of length τ_0 in the open states. An alternative assumption is that transfer is detected immediately, with the provision that only sojourns of length at least τ_0 are detected. An analogous framework to ours can be constructed if the above alternative assumption is appropriate. The only difference is that J_k now corresponds to the state that the channel is in at the start of the $(k+1)$st detected sojourn, rather than after a time lapse of τ_0. Corresponding expressions for $\Phi(\theta)$ can be derived but they tend to be slightly more complicated than before. The unconditional moments and autocorrelation functions will be the same for the two forms of time interval omission. However, this would not be the case if τ_0 was different for open and closed sojourns, though the differences can easily be determined by elementary methods. When $\tau_0 = 0$ the two forms of time interval omission obviously become identical. The resulting embedded process $\{(J_k, T_k); k = 0, 1, \ldots\}$ provides an illuminating way of analyzing model channel gating kinetics when time interval omission is absent. Initially, our form for time interval omission was chosen for mathematical convenience. However, it also corresponds closely to what happens in the physical situation. For example, Colquhoun and Sigworth (1983) have suggested that a consistent minimum sojourn time (τ_0) be imposed on the idealized data after the latter has been derived from the experimental record.

Roux and Sauvé (1985) derived a complex integral expression for the unconditional pdf of observed open (closed) sojourn times incorporating time interval omission. We can derive a similar expression from our results as follows. Let $f_o(t)$ $(t > 0)$ be the pdf of observed open sojourn times, with Laplace transform

$$\phi_o(\theta) = \int_0^{\infty} \exp(-\theta t) f_o(t)\, dt \quad (\theta \geq 0).$$

Then it is readily shown that

$$\phi_o(\theta) = \sum_{i=1}^{n_0} \sum_{j=n_0+1}^{n} \pi_i^o \phi_{ij}(\theta) \quad (\theta \geq 0). \tag{23}$$

Setting $\theta = -iu$ in Eqs. 23 and 3 we obtain the characteristic function of observed open sojourn times (Fourier transform of $f_o(t)$). Application of the Fourier inversion theorem (see, e.g., Grimmett and Stirzaker, 1982) yields

$$f_o(t) = \frac{1}{2\pi} \int_{-\infty}^{\infty} \exp(iut) \phi_o(iu)\, du \quad (t > 0), \tag{24}$$

and similarly for closed sojourns.

The Eq. 24, after substitution from Eqs. 23 and 3, differs from the equivalent formula in Roux and Sauvé (1985), owing to the equilibrium behavior of the observed process being modeled differently. Roux and Sauvé (1985) do not explicitly model the observed dynamic process, but rather consider an observed sojourn in isolation. For example, they assume that an observed open sojourn is immediately preceded by an actual closed sojourn of length at least τ_0 and condition on the equilibrium behavior of the initial state of such a closed sojourn. However, their assumption is not precisely correct, since observed open sojourns can be preceded by actual closed sojourns of length $<\tau_0$, for example, the final observed open sojourn in Fig. 1. Thus Roux and Sauvé's results differ from ours, but in most practical situations the difference is likely to be very small.

In practice, numerical methods will be required to invert the matrix Laplace transform $\Phi(\theta)$ to obtain the matrix function $F(t)$. This can in principle be achieved by use of a fast Fourier transform, but convergence problems may arise. Further research is required to find a numerical method of inverting $\Phi(\theta)$, that is computationally feasible for a wide class of models (Roux and Sauvé, 1985; Ball and Sansom, 1988). The lack of a closed form expression for $F(t)$ is no great hinderance from a mathematical viewpoint, since knowing $\Phi(\theta)$ for all $\theta \geq 0$ is formally equivalent to knowing $F(t)$ for all $t > 0$. Also, as we have seen, several important properties of observed channel kinetics, such as moments and autocorrelation functions, can be derived directly from $\Phi(\theta)$. However, an efficient numerical inversion of $\Phi(\theta)$ would undoubtedly be useful. It would enable us to display model pdf's of observed sojourn times. More importantly, it would also enable a closer examination of the effects of time interval omission on current methods for determining the numbers of open and closed states from the forms of such sojourns time pdf's.

We turn now to the analysis of sojourn time autocorrelation functions in the presence of time interval omission. We have shown that, provided the entry process transition matrices, P_o^J and P_c^J, are diagonalisable with positive real eigenvalues, the autocorrelation functions, $\Gamma_o(k)$ and $\Gamma_c(k)$, are still each made up of $\leq m - 1$ geometrically decaying terms when time interval omission is incorporated. Thus inferences made in the absence of time interval omission will still be valid. We reiterate that, as yet, we are unable to provide a proof that the matrices P_o^J and P_c^J necessarily possess the above mentioned properties, though they have in all of our numerical studies. If, as seems unlikely, for a particular model the matrices P_o^J or P_c^J do not possess the above properties then it is also unlikely that the autocorrelation functions $\Gamma_o(k)$ and $\Gamma_c(k)$ may be expressed as weighted sums of geometrically decaying terms. Thus, if estimated autocorrelation functions from observed single channel kinetics are well approximated by weighted sums of geometrically decaying terms, then it suggests that the matrices P_o^J and P_c^J do indeed possess the required properties, so appropriate inferences will be made.

Further research is required into methods of estimating the number of geometrically decaying components, m' say, from observed autocorrelation functions. It is perhaps worth noting that, in our preliminary investigation using simulated data described earlier, m' was always underestimated. This is not too serious since, in any case, m' is a lower bound for m. This would not be the case if m' was being overestimated, as that might lead to more complicated models than necessary being used.

Finally, we re-emphasize that we have shown theoretically that successive observed sojourns are necessarily uncorrelated if $m = 1$, even in the presence of time interval omission. Thus, in the situation when a model with $m = 1$ is hypothesized, experimentally observed nonzero correlations are indicative of the model being too simple. They are not an artifact of time interval omission, and hence one may interpret the results of autocorrelation analysis with some degree of confidence.

APPENDIX

In the appendix we show that if in the absence of time interval omission the lengths of successive observed open sojourns are uncorrelated, owing to the transition matrix $P_o^J(0)$ having rank one, then they remain uncorrelated when time interval omission is incorporated. We shall require the following lemma.

Lemma: Let A be an $m \times n$ matrix. Then rank $(AA^\tau) = 1$ implies rank $(A) = 1$.

Proof: The result is trivial if $A = 0$ so suppose that $A \neq 0$. Let $(x_1, x_2, \ldots, x_n)$ and $(y_1, y_2, \ldots, y_n)$ be the first two rows of A. We can assume without loss of generality that $\Sigma_{i=1}^n x_i^2 \neq 0$. The top left 2×2 submatrix of AA^τ is

$$\begin{bmatrix} \sum_{i=1}^n x_i^2 & \sum_{i=1}^n x_i y_i \\ \sum_{i=1}^n x_i y_i & \sum_{i=1}^n y_i^2 \end{bmatrix}.$$

The above matrix has zero determinant, since rank $(AA^\tau) = 1$, so

$$\sum_{i=1}^n x_i^2 \sum_{i=1}^n y_i^2 = \left(\sum_{i=1}^n x_i y_i \right)^2.$$

It follows from the condition for equality in Cauchy's inequality that there exists a real number, θ say, such that

$$y_i = \theta x_i \quad (i = 1, 2, \ldots, n).$$

Thus the second row of A is a multiple of the first row. A similar argument shows that all the rows of A are a multiple of the first row, so A has rank one as required.

It follows from Eq. 4 and the definition of $P_o^J(\tau_0)$ that

$$P_o^J(\tau_0) = [Q_{oo} - Q_{oc}Q_{cc}^{-1}\{I - \exp(\tau_0 Q_{cc})\}Q_{co}]^{-1}Q_{oc}\exp(\tau_0 Q_{cc}) \cdot [Q_{cc} - Q_{co}Q_{oo}^{-1}\{I - \exp(\tau_0 Q_{oo})\}Q_{oc}]^{-1}Q_{co}\exp(\tau_0 Q_{oo}). \quad \text{(A1)}$$

Setting $\tau_0 = 0$ in Eq. A1 we obtain

$$P_o^J(0) = Q_{oo}^{-1}Q_{oc}Q_{cc}^{-1}Q_{co}.$$

A standard result from linear algebra states that if A is a nonsingular square matrix then

$$\text{rank}\,(AB) = \text{rank}\,(BA) = \text{rank}\,(B), \quad \text{(A2)}$$

provided the products are defined.

Partition the diagonal matrix W of equilibrium probabilities for the underlying process $\{X(t); t \geq 0\}$ into

$$W = \begin{bmatrix} W_o & \mathbf{0} \\ \mathbf{0} & W_c \end{bmatrix}.$$

Then, by repeated application of Eq. A2, it follows that

$$\begin{aligned} \text{rank}\,[P_o^J(0)] &= \text{rank}\,(Q_{oo}^{-1}Q_{oc}Q_{cc}^{-1}Q_{co}) \\ &= \text{rank}\,(Q_{oc}Q_{cc}^{-1}Q_{co}) \\ &= \text{rank}\,(W_o^{1/2}Q_{oc}W_c^{-1/2}W_c^{1/2}Q_{cc}^{-1}W_c^{-1/2} \\ &\quad \cdot W_c^{1/2}Q_{co}W_o^{-1/2}) \\ &= \text{rank}\,(W_o^{1/2}Q_{oc}W_c^{-1/2}W_c^{1/2}(-Q_{cc}^{-1}) \\ &\quad \cdot W_c^{-1/2}W_c^{1/2}Q_{co}W_o^{-1/2}). \end{aligned} \quad \text{(A3)}$$

It follows from Eq. 20 that $W_c^{1/2}(-Q_{cc}^{-1})W_c^{-1/2}$ is symmetric and hence can be written in the form

$$W_c^{1/2}(-Q_{cc}^{-1})W_c^{-1/2} = HDH^{\tau},$$

where H is orthogonal and D is a diagonal matrix containing the eigenvalues of $-Q_{cc}^{-1}$, which are all strictly positive (Ball and Sansom, 1988). Thus $W_c^{1/2}(-Q_{cc}^{-1})W_c^{-1/2}$ may be written as

$$W_c^{1/2}(-Q_{cc}^{-1})W_c^{-1/2} = HD^{1/2}H^{\tau}HD^{1/2}H^{\tau},$$

so

$$\text{rank}\,[P_o^J(0)] = \text{rank}\,(W_o^{1/2}Q_{oc}W_c^{-1/2}HD^{1/2} \cdot H^{\tau}HD^{1/2}H^{\tau}W_c^{1/2}Q_{co}W_o^{-1/2}).$$

Now it follows from Eq. 21 that

$$W_o^{1/2}Q_{oc}W_c^{-1/2} = (W_c^{1/2}Q_{co}W_o^{-1/2})^{\tau}, \quad \text{(A4)}$$

so

$$\text{rank}\,[P_o^J(0)] = \text{rank}\,(AA^{\tau}),$$

where

$$A = W_o^{1/2}Q_{oc}W_c^{-1/2}HD^{1/2}H^{\tau}.$$

Thus, by the Lemma, rank $[P_o^J(0)] = 1$ implies rank $(A) = 1$ and repeated application of Eq. (A2) yields

$$\text{rank}\,[P_o^J(0)] = 1 \rightarrow \text{rank}\,(Q_{oc}) = 1.$$

Now from Eq. A4

$$Q_{oc} = W_o^{-1}Q_{co}^{\tau}W_c$$

so, using Eq. A2,

$$\begin{aligned} \text{rank}\,(Q_{oc}) &= \text{rank}\,(Q_{co}^{\tau}) \\ &= \text{rank}\,(Q_{co}). \end{aligned}$$

Another result from linear algebra states that

$$\text{rank}\,(AB) \leq \min\,[\text{rank}\,(A), \text{rank}\,(B)].$$

Repeated application of this to Eq. A1 yields

$$\text{rank}\,[P_o^J(\tau_0)] \leq \min\,[\text{rank}\,(Q_{oc}), \text{rank}\,(Q_{co})].$$

Thus

$$\text{rank}\,[P_o^J(0)] = 1 \rightarrow \text{rank}\,[P_o^J(\tau_0)] = 1 \text{ for all } \tau_0 > 0,$$

since the rank of any matrix must be at least one.

It is straightforward to deduce from Eq. A1 that

$$P_c^J(0) = Q_{cc}^{-1}Q_{co}Q_{oo}^{-1}Q_{oc},$$

and a similar argument to the above shows that rank $[P_o^J(0)] = 1$ if and only if rank $[P_c^J(0)] = 1$.

Now suppose that rank $[P_o^J(0)] = 1$ and $\tau_0 > 0$. Clearly each element of $P_o^J(\tau_0)$ will be strictly positive so, since the rows of $P_o^J(\tau_0)$ are proportional to each other and each sum to unity, they must be identical and necessarily equal to the equilibrium distribution π_o^{τ} of the open entry process. It follows that $P_o^J(\tau_0)$ admits the spectral representation (9), with $m = 1$, so successive observed open sojourns are uncorrelated. A similar argument holds for closed sojurns, and also for the case $\tau_0 = 0$ provided we restrict attention to gateway states.

GLOSSARY

- n number of states in the continuous time Markov chain, $\{X(t);\ t \geq 0\}$, describing single channel gating kinetics
- n_o number of open states
- n_c number of closed states
- Q infinitesimal transition rate matrix for underlying

continuous time Markov chain, $\{X(t);\ t \geq 0\}$. Q is partitioned into

$$\begin{bmatrix} Q_{oo} & Q_{oc} \\ Q_{co} & Q_{cc} \end{bmatrix}$$

$$Q_0 \begin{bmatrix} Q_{oo} & 0 \\ 0 & Q_{cc} \end{bmatrix}.$$

$$Q_1 \begin{bmatrix} 0 & Q_{oc} \\ Q_{co} & 0 \end{bmatrix}$$

π equilibrium distribution of $\{X(t);\ t \geq 0\}$

τ_0 minimum detectable sojourn

P^J transition matrix of embedded Markov chain $\{J_k;\ k = 0, 1, \ldots\}$. J_k = state $\{X(t);\ t \geq 0\}$ is in when the kth sojourn is detected.

P^J_o transition matrix of open entry process, $\{J_{2k};\ k = 0, 1, \ldots\}$

P^J_c transition matrix of closed entry process, $\{J_{2k+1};\ k = 0, 1, \ldots\}$

π_o equilibrium distribution of open entry process

π_c equilibrium distribution of closed entry process

$M^{(r)}$ matrix pertaining to rth moments of observed sojourns

$\mu^{(r)}$ $\mu^{(r)} = (\mu_1^{(r)}, \mu_2^{(r)}, \ldots, \mu_n^{(r)})^T$, where T denotes transpose. For $i = 1, 2, \ldots, n_o$, $\mu_i^{(r)}$ is the rth moment of observed open sojourns with entry state i. For $i = n_o + 1, n_o + 2, \ldots, n$, $\mu_i^{(r)}$ is the rth moment of observed closed sojourns with entry state i.

$\mu_o^{(r)}$ unconditional rth moment of observed open sojourns

$\mu_c^{(r)}$ unconditional rth moment of observed closed sojourns

m_o number of open gateway states

m_c number of closed gateway states

m $\min\{m_o, m_c\}$

$\Gamma_o(k)$ autocorrelation function for observed open sojourns

$\Gamma_c(k)$ autocorrelation function for observed closed sojourns

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