Single ion channel models incorporating aggregation and time interval omission

Frank G. Ball,* Geoffrey F. Yeo,‡ Robin K. Milne,§ Robert O. Edeson,¶ Barry W. Madsen,‖ and Mark S. P. Sansom**

*Department of Mathematics, University of Nottingham, NG7 2RD, United Kingdom; ‡School of Mathematical and Physical Sciences, Murdoch University, Murdoch, 6150, Western Australia; §Department of Mathematics and ‖Department of Pharmacology, University of Western Australia, Nedlands, 6009, Western Australia; ¶Department of Anaesthesia, Sir Charles Gairdner Hospital, Nedlands, 6009, Western Australia; and **Laboratory of Molecular Biophysics, University of Oxford, OX1 3QU, United Kingdom

ABSTRACT We present a general theoretical framework, incorporating both aggregation of states into classes and time interval omission, for stochastic modeling of the dynamic aspects of single channel behavior. Our semi-Markov models subsume the standard continuous-time Markov models, diffusion models and fractal models. In particular our models allow for quite general distributions of state sojourn times and arbitrary correlations between successive sojourn times. Another key feature is the invariance of our framework with respect to time interval omission: that is, properties of the aggregated process incorporating time interval omission can be derived directly from corresponding properties of the process without it. Even in the special case when the underlying process is Markov, this leads to considerable clarification of the effects of time interval omission. Among the properties considered are equilibrium behavior, sojourn time distributions and their moments, and auto-correlation and cross-correlation functions. The theory is motivated by ion channel mechanisms drawn from the literature, and illustrated by numerical examples based on these.

INTRODUCTION

The direct observation of single channel activity using the patch clamp technique has stimulated the development of stochastic models as the basis for inference about ion channel kinetics from experimental data. For this purpose the channel molecule is generally considered to be, at any time, in one of a finite number of kinetically (physico-chemically) distinct states linked by well defined transition pathways. These states are represented by the state space of the stochastic process with which the channel kinetics are modelled.

In practice, the elucidation of channel characteristics is hindered by the fact that transitions between actual states are, in (at least) two senses, only partially observable. First, transitions can be seen only if they occur between states having different conductance, since it is this (or equivalently, ionic current) which the patch clamp technique records. Thus the state space of the observable stochastic process corresponds to condensations or aggregations of actual states into classes that are experimentally distinguishable, these being usually just conducting (open) and nonconducting (closed). Second, very short sojourn times which may in principle be observable in the above sense may not be seen because of limitations in the electronic recording system and the need for filtering. This limited time resolution or time interval omission further degrades the observed process as a representation of actual transitions. If the purpose of inference is to characterise the channel from its observable behavior, it follows that the stochastic models subserving it must take account of the limits to observability; that is, incorporate aggregation and time interval omission.

In this paper we present a general mathematical framework, encompassing both of the above phenomena, for stochastic modeling of a complete temporal record of a single ion channel having arbitrary time-invariant kinetics. Our approach is based on semi-Markov processes on arbitrary finite state spaces, and allows quite general distributions for sojourn times in individual states, together with arbitrary correlations between successive sojourn times. It provides a unified setting which includes the standard continuous-time Markov models (Colquhoun and Hawkes, 1982; Fredkin et al., 1985; Ball and Sansom, 1988*a*), alternating renewal models (Milne et al., 1988), fractal models (Liebovitch et al., 1987) and diffusion models (Millhauser et al., 1988*a*; Läuger, 1988) as special cases. In particular, Ball and Sansom (1988*a*) assumed the underlying single channel process was a continuous-time Markov chain and derived properties of the aggregated process incorporating time interval omission; Milne et al. (1988) used alternating renewal models for the aggregated process both with and without time interval omission. Roux and Sauvé (1985) and Blatz and Magleby (1986) had earlier studied aspects of time interval omission. An advantage of the semi-Markov framework is that it is invariant to time interval omission: by this we mean that if the aggregated process not incorporating time interval omission is semi-Markov (as is the case for almost all the models considered in the literature) then so is the aggregated process incorporating time interval omission. This invariance property provides clarification of the effects of time interval omission even when the underlying process is Markov, and leads to considerable economy in the derivation of single channel properties.

The paper is divided broadly into two parts, covering theory and applications. The theory part begins with a description of underlying assumptions, and then uses illustrative examples to motivate the semi-Markov approach and the various processes involved. It summarizes key ideas about semi-Markov processes and indicates how the previous approaches result as special cases. Then follows a development of results giving channel

0006-3495/93/02/357/18 $2.00

properties, such as equilibrium behavior, densities, means, correlations and other moments of sojourn times, for the aggregated process without time interval omission associated with a given underlying semi-Markov process. Results for the aggregated process with time interval omission are then obtained from expressions in terms of corresponding properties for the process without time interval omission. The framework is then specialized to the Markov case and key formulae presented in a table. Our approach is based on that of Ball et al. (1991), although the presentation here places greater emphasis on examples, these being used both to motivate and illustrate the theory. Reference should be made to Ball et al. (1991) for detailed derivations.

The applications part describes some computational considerations that form the basis of computer programs we have employed to implement calculations using the formulae presented earlier, and gives numerical examples, both Markov and non-Markov, based on these calculations.

We conclude with a discussion assessing the contribution of the semi-Markov approach, and focusing on some remaining problems. An appendix outlines a method, based on integral equations, for numerical calculation of certain moments required in the presence of time interval omission.

THEORY

Assumptions concerning physical properties of ion channels

Before proceeding with a mathematical formulation of ion channel gating models, it is valuable to consider the underlying physical assumptions. It is important to stress that these assumptions are made in the absence of information about the three dimensional structure and molecular motions of ion channel proteins. However, analogy with the dynamic properties of globular proteins (Brooks et al., 1988) provides a basis for understanding ion channels. The principal assumptions are as follows:

(*a*) Ion channel gating mechanisms may be described in terms of a finite number of discrete, well defined states of the channel. That the number of states is finite seems inherently reasonable, although the number of conformational states of the channel protein may be very large. By well defined it is meant that the kinetic states detectable via single channel analysis correspond to distinct minima on the conformational energy surface of the channel protein. Protein conformational states and substates have been discussed, for example by Frauenfelder et al. (1988), and these may be readily incorporated into the general class of gating models discussed in this paper.

(*b*) The gating mechanism is time invariant. At first this seems somewhat restrictive, as it has been shown on several occasions that state-switching of channel gating occurs (e.g., Moczydlowski, 1986). However, if one assumes that such behavior corresponds to (relatively infrequent) modification of channel structures by, for example phosphorylation/dephosphorylation (Levitan, 1988), then it would be fairly straightforward to include such processes in the overall kinetic model. More complex models (Croxton, 1988) however, cannot be readily included.

(*c*) Switching between states is assumed to be an inherently stochastic process, with the channel retaining no memory of its previous states (but, unlike in Markov models, memory of the time since the last state transition may be kept). In the absence of any evidence for such memory effects, this assumption seems to be reasonable.

Although not directly required in the general theory, an assumption of time reversibility leads to useful computational simplifications. Time reversibility is equivalent to assuming that channel gating is not coupled to a source of free energy, such as ion concentration gradients across the membrane. This assumption has been discussed in relation to channel gating by Läuger (1983).

Having stated the assumptions, it is useful to examine the extent to which these apply in contemporary models of channel gating. They clearly are satisfied by the classical Markov models of channel gating (e.g., McManus and Magleby, 1989; Sansom et al., 1989). They also may be applied to recently discussed diffusion models of channel gating. Several of these are Markov models with large state spaces (Läuger, 1988, Millhauser et al., 1988*a*, *b*; Oswald et al., 1991), and thus clearly come within the current frame of reference. More recently, defect diffusion models of channel gating have been discussed (Condat, 1989; Condat and Jäckle, 1989). It seems likely that these can be recast in a form suitable for analysis using the methods described below. Similarly, fractal gating models (Liebovitch and Sullivan, 1987) may also be incorporated. There are some models, however, which do not fit into the framework used in this paper. In particular, it is difficult to see how the current methods could be used to analyze the continuum model of Levitt (1989), or the fine structure of the percolation (Doster et al., 1990) or reptation (Millhauser, 1990) models of channel gating. However, it appears that both these latter two models lead to successive open and closed sojourns being modelled by an alternating renewal process, and hence their broad structure is covered by our theory.

Motivation for semi-Markov framework

We begin with a five-state example

$$\begin{array}{ccccc} C_5 & \rightleftarrows & C_4 & \rightleftarrows & C_3 \\ & & \upharpoonleft\downharpoonright & & \upharpoonleft\downharpoonright \\ & & O_1 & \rightleftarrows & O_2 \end{array} \qquad (1)$$

frequently considered in the literature as a mechanism for an agonist-gated (e.g., nicotinic acetylcholine receptor) channel. In such a mechanism open (unit conductance) states are indicated by O, and closed (zero conductance) states by C, with subscripts indicating the state label as used in subsequent models. In Eq. 1 there are four gateway states, that is states which can be entered directly from a state of the other type, two open (1 and 2) and two closed (3 and 4). Note that this definition is different from that adopted by Colquhoun and Hawkes (1987). Some other mechanisms that have been considered in the literature are:

$$\begin{array}{ccccccc} C_7 & \rightleftarrows & C_6 & \rightleftarrows & C_5 & \rightleftarrows & C_4 \\ & & & & \upharpoonleft\downharpoonright & & \upharpoonleft\downharpoonright \\ & & & & O_1 & & O_2 \rightleftarrows C_3 \end{array} \quad (2)$$

and

$$\begin{array}{ccc} C_3 & \rightleftarrows & C_4 \\ \nearrow\swarrow & & \searrow\nwarrow \\ O_2 & \rightleftarrows & O_1 \end{array} \quad (3)$$

In Eq. 2, a scheme considered for the chloride channel by Blatz and Magleby (1989), the open gateway states are 1 and 2 and the closed gateway states 3, 4, and 5; in Eq. 3, a variant of a scheme considered by Colquhoun and Hawkes (1987), the gateway states are 1, 2, and 3.

The usual stochastic models based on such mechanisms are continuous-time homogeneous Markov chains with the appropriate number of states. For example, the five-state model based on Eq. 1 has been discussed in detail by Colquhoun and Hawkes (1982). In such a model transitions between states are governed by a (homogeneous) discrete-time Markov chain (the jump chain), and given the successive states visited the sojourn times in individual states are independent, having exponential distributions with parameters that depend only on the state being visited.

For such models the underlying (homogeneous continuous-time) Markov chain, denoted by $\{X(t);\, t \geq 0\}$, which records the state $X(t)$ that the channel is in at each time t, is unobservable; in the absence of time interval omission it is the aggregated process (see, e.g., Fredkin et al., 1985), recording which of certain classes of states the underlying process is in at each time, that is in principle observable. In models based on the above schemes there are two classes of states; for the five-state Markov model $\mathcal{O} = \{1, 2\}$ and $\mathcal{C} = \{3, 4, 5\}$, denoting classes of open and closed states, respectively. Complete information about the aggregated process is contained in the corresponding gateway process $\{(J_k, T_k);\, k = 0, 1, \cdots\}$, where J_k, $k = 0, 1, \cdots$ are the gateway or entry states, and T_k, $k = 1, 2, \cdots$ the sojourn times for successive sojourns in the classes $\mathcal{O}$ and $\mathcal{C}$ and, for completeness, $T_0 = 0$. Fig. 1 illustrates typical processes associated with the above five-state Markov model. Because the entry states cannot be distinguished, the gateway process is unobservable; it simply provides an appropriate intermediate process by means of which properties of the aggregated process can be derived. Throughout the paper we write, for example, $\{X(t)\}$ instead of $\{X(t);\, t \geq 0\}$, and $\{(J_k, T_k)\}$ for $\{(J_k, T_k);\, k = 1, 2, \cdots\}$.

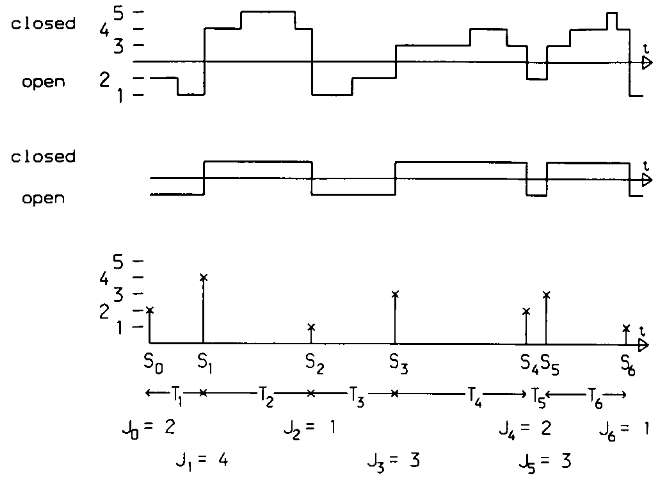

FIGURE 1 Illustration of typical processes associated with the five-state Markov model (Eq. 1). The top diagram is a realisation of the underlying (unobservable) Markov chain $\{X(t);\, t \geq 0\}$. The middle diagram shows the corresponding realisation of the aggregated process (without time interval omission) which is in principle observable. The final diagram illustrates the corresponding realisation of the gateway process $\{(J_k, T_k);\, k = 0, 1, \cdots\}$.

Although for any Markov model $\{X(t)\}$, the gateway process $\{(J_k, T_k)\}$ inherits the Markov property it is not in general a continuous-time Markov chain. Rather, the gateway states are visited according to the (homogeneous discrete-time) Markov chain $\{J_k\}$ which we call the entry process and, given the states so visited, the sojourn times T_k, $k = 1, 2, \cdots$ are conditionally independent with the distribution of T_k depending only on the state J_{k-1} currently being visited and the state J_k that is subsequently visited. Such a process $\{(J_k, T_k)\}$ is essentially a Markov renewal or semi-Markov process (Çinlar, 1969 and 1975, Chapter 10; Pyke, 1961): the equivalent process $\{(J_k, S_k)\}$, where $S_0 = 0$ and $S_k = \sum_{i=1}^{k} T_i$ for each positive integer k, is precisely a Markov renewal process. If the channel were in the open class of states at time $S_0 = 0$ then S_1 gives the time at which the channel first enters the closed class, S_2 the time at which it next enters the open class, and so on. Another equivalent process is $\{Y(t)\}$ defined for each t in the time interval $S_k \leq t < S_{k+1}$ by $Y(t) = J_k\, (k = 0, 1, \cdots)$, and this is a semi-Markov process. Its value $Y(t)$ at any time t gives the entry state of the current class sojourn. Clearly the above descriptions are equivalent, and for ease of exposition we call $\{(J_k, T_k)\}$ a semi-Markov process. Thus our semi-Markov model assumes that the channel moves between a finite number of states. The sojourn times in each state follow arbitrary but specified distributions. On entering a new state the sojourn time in that state is independent of the previous history of the channel, though it depends on the state currently being visited. On leaving that state, the next state visited by the channel can depend on both the state the channel has just left and the time spent there. In contrast, Markov models of channel gating assume that (a) the sojourn times of the channel in different states follow exponential distributions and (b) the next state visited by the channel depends only on the previous state, and not on the time spent there.

In practice, even the aggregated process is not fully observable: brief sojourns in one or other of the classes $\mathcal{O}$ and $\mathcal{C}$ may fail to be detected. We suppose (see also Colquhoun and Sigworth, 1983; Ball and Sansom, 1988a; and Milne et al., 1988) that any sojourn, open or closed, is detected if and only if its duration is greater than some fixed detection limit (dead-time) τ. Then for example, because consecutive openings separated by undetected closures will appear to be a single opening, the observable open-time distribution (in the aggregated process incorporating so-called time interval omission) will differ from the true open-time distribution (in the aggregated process). Formally, an observable open-time is defined to begin with a (true) open-time greater than the detection limit τ, this being followed by open-times not necessarily greater than τ which are separated by closed-times each having length at most τ. The start of the first subsequent closed-time greater than τ signals the end of the observable open-time and the start of the following observable closed-time. An observable closed-time is defined similarly.

Just as complete information about the aggregated process is contained in its gateway process $\{(J_k, T_k)\}$, so the aggregated process incorporating time interval omission is completely described by a corresponding gateway process $\{(\tilde{J}_k, \tilde{T}_k)\}$, where $\tilde{J}_k$, $k = 0, 1, \cdots$ are the gateway or entry states for observable sojourns, $\tilde{T}_k$, $k = 1, 2, \cdots$ the successive observable class sojourn times, and $\tilde{T}_0 = 0$. Fig. 2 illustrates the gateway processes associated with the five-state Markov model (Eq. 1). The gateway process $\{(\tilde{J}_k, \tilde{T}_k)\}$ is another semi-Markov process. Thus gateway states are visited according to the entry process $\{\tilde{J}_k\}$ which is a Markov chain and, given the states so visited, the sojourn times $\tilde{T}_k$, $k = 1, 2, \cdots$ are (conditionally) independent with the distribution of $\tilde{T}_k$ depending only on the state $\tilde{J}_{k-1}$ currently being visited and the state $\tilde{J}_k$ subsequently visited.

For Markov models the gateway process $\{(J_k, T_k)\}$ is not a continuous-time Markov chain except in certain circumstances, such as when there is exactly one open and one closed state. Hence, to deal with gateway processes arising from even the traditional Markov models for a single ion channel (Colquhoun and Hawkes, 1982; Fredkin et al., 1985; Ball and Sansom, 1988a) we are led to semi-Markov gateway processes. Furthermore, this class of gateway processes covers also the (non-Markov) alternating renewal models discussed in Liebovitch et al. (1987) and Milne et al. (1988). Although such non-Markov models allow for arbitrary distributions of state

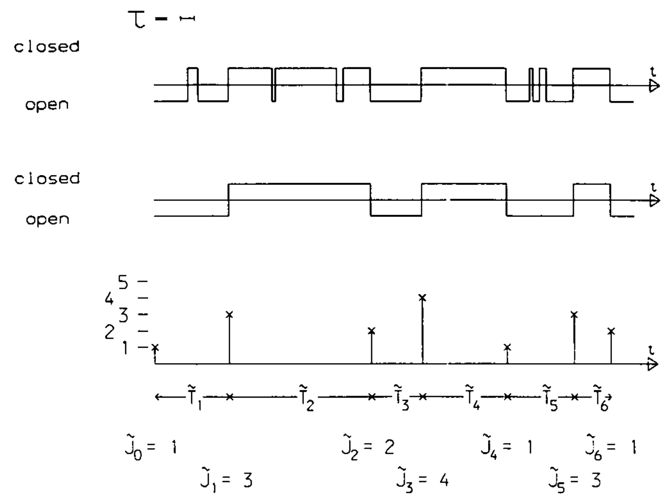

FIGURE 2 Illustration of processes incorporating time interval omission associated with the five-state Markov model (Eq. 1). The top diagram is a realisation of the aggregated process without time interval omission, and shows the size of the detection limit τ. In the middle diagram is the corresponding realisation of the aggregated process with time interval omission, and its gateway process $\{(\tilde{J}_k, \tilde{T}_k);\ k = 0, 1, \cdots\}$ is illustrated in the bottom diagram.

sojourn times, they do not allow non-zero correlations between sojourn times, and these are often obtained in single channel data (see, e.g., Labarca et al., 1985; McManus et al., 1985; Kerry et al., 1987). By contrast, semi-Markov models permit arbitrary correlations between successive sojourn times. Thus the semi-Markov framework is sufficiently rich that it can encompass and extend so many of the commonly used single channel models. As we now describe, an additional feature of this framework is its invariance under time interval omission.

As the gateway processes contain the minimum amount of information needed in order to derive channel properties, the semi-Markov framework is the natural mathematical setting for stochastic modeling of a single ion channel. The invariance property, or the parallel between the aggregated process without time interval omission and its gateway process $\{(J_k, T_k)\}$ on the one hand, and the aggregated process with time interval omission and its gateway process $\{(\tilde{J}_k, \tilde{T}_k)\}$ on the other, enhances the importance of the semi-Markov theory. Finally, having noted that the generality of the semi-Markov framework is needed even in the traditional setting when the underlying process $\{X(t)\}$ is a Markov chain, we observe that when the underlying process is itself assumed to be semi-Markov the resultant gateway processes are necessarily also semi-Markov. That is, the semi-Markov theory offers a natural and significant generalization of the standard Markov theory. (In any case, the semi-Markov setting is really the most general possible given stochastic process theory as presently developed.) Furthermore, the semi-Markov framework allows clarification of the role of time interval omission and considerable economy in the derivation of single channel properties such as equilibrium distributions and sojourn time probability density functions (pdfs), means (expectations), correlations and other sojourn time moments. This is achieved by expressing such properties explicitly in terms of parameters of gateway processes (and thereby parameters of the underlying process). In particular, this allows time interval omission to be incorporated with minimal effort once results have been derived without time interval omission.

The gateway process and observed channel properties

Suppose that there are m_o open gateway states (i.e., open states that the channel can reach directly from closed states), m_c closed gateway states, and let $m = m_o + m_c$. Label the gateway states $a_1, a_2, \ldots, a_m$ where $\mathcal{O}_G = \{a_1, a_2, \ldots, a_{m_o}\}$ and $\mathcal{C}_G = \{a_{m_o+1}, a_{m_o+2}, \ldots, a_m\}$ are the open and closed gateway states, respectively. Suppose that the channel enters the open states at time $t = 0$. Let S_1 be the time at which the channel first enters a closed state, S_2 be the time at which the channel next enters an open state and so on. Set $S_0 = 0$ and for $k = 0, 1, \cdots$ let J_k be the state the channel enters at time S_k. Thus J_k belongs to $\mathcal{O}_G$ for k even and $\mathcal{C}_G$ for k odd. Set $T_0 = 0$ and for $k = 1, 2, \cdots$ let $T_k = S_k - S_{k-1}$. Thus T_1 is the length of the first open sojourn, T_2 the length of the first closed sojourn and so on. The observed behavior of the single ion channel, without time interval omission, is contained in the process $\{(J_k, T_k)\}$ (see Fig. 1) which we assume to be semi-Markov.

The properties of $\{(J_k, T_k)\}$ are then completely determined by the $m \times m$ matrix function $\mathbf{F}(t) = [F_{ij}(t)]$, known as the semi-Markov kernel, defined elementwise by

$$F_{ij}(t) = \mathbb{P}(J_k = a_j \text{ and } T_k \le t \mid J_{k-1} = a_i) \quad (t \ge 0; i, j = 1, 2, \ldots, m).$$

Thus if $a_i \in \mathcal{O}_G$ and $a_j \in \mathcal{C}_G$, $F_{ij}(t)$ is the probability that an open sojourn starting in state a_i has duration at most t and on leaving the open states the channel goes to the closed state a_j. Note that since $\{J_k\}$ alternates between open and closed states, $\mathbf{F}(t)$ may be partitioned into

$$\mathbf{F}(t) = \begin{bmatrix} \mathbf{0} & \mathbf{F}_{oc}(t) \\ \mathbf{F}_{co}(t) & \mathbf{0} \end{bmatrix}.$$

Here $\mathbf{F}_{oc}(t)$ is an $m_o \times m_c$ matrix function corresponding to open sojourns and $\mathbf{F}_{co}(t)$ an $m_c \times m_o$ matrix function corresponding to closed sojourns. Throughout the paper $\mathbf{0}$ denotes a zero matrix (or vector), whose dimension is apparent from the context. It follows that

$$\mathbf{P}^J = \mathbf{F}(\infty)$$

is the transition matrix of the Markov chain $\{J_k\}$ which records the state the channel is in each time an open or closed sojourn commences. Let $\mathbf{f}(t) = [f_{ij}(t)]$ be the $m \times m$ matrix function given by

$$\mathbf{f}(t) = \mathbf{F}'(t) \quad (t > 0),$$

where the differentiation is elementwise, i.e., $f_{ij}(t) = F'_{ij}(t)$. Throughout the paper differentiation and integration of matrix functions are defined in this elementwise sense. The matrix function $\mathbf{f}(t)$ contains, as we later show, information from which the probability density functions (pdfs) of open and closed sojourns can be obtained.

In practice, a closed form expression for $\mathbf{f}(t)$ may not be available, and we shall often work in terms of its Laplace transform $\Phi(\theta) = [\Phi_{ij}(\theta)]$ given by

$$\Phi(\theta) = \int_0^\infty e^{-\theta t} \mathbf{f}(t)\, dt \quad (\theta \ge 0).$$

Note that $\mathbf{P}^J = \Phi(0)$.

In order to determine unconditional channel properties we require the equilibrium behavior of the channel. The entry process $\{J_k\}$ alternates between open and closed states, so will not possess an equilibrium distribution. Instead consider the process $\{J_{2k}; k = 0, 1, \cdots\}$, from here on denoted by $\{J_{2k}\}$, which we term the open entry process as it records the state the channel is in each time an opening occurs. The $m_o \times m_o$ transition matrix, $\mathbf{P}^J_o = [(\mathbf{P}^J_o)_{ij}]$ say, of the open entry process is given by

$$\mathbf{P}^J_o = \mathbf{P}^J_{oc}\mathbf{P}^J_{co}, \tag{5}$$

where $\mathbf{P}^J$ has been partitioned in the same fashion as Eq. 4. The open entry process possesses an equilibrium distribution, $\pi^o = (\pi^o_1, \pi^o_2, \ldots, \pi^o_{m_o})^\top$ say (where $^\top$ denotes transpose), which can be obtained by solving the linear equations

$$\sum_{j=1}^{m_o} \pi^o_j (\mathbf{P}^J_o)_{ji} = \pi^o_i \quad (i = 1, 2, \ldots, m_o - 1) \tag{6}$$

and

$$\sum_{j=1}^{m_o} \pi^o_j = 1. \tag{7}$$

Similarly we define the closed entry process $\{J_{2k+1}; k = 0, 1, \cdots\}$, hereafter denoted by $\{J_{2k+1}\}$, which records the state the channel is in each time a closure occurs. It has an $m_c \times m_c$ transition matrix, $\mathbf{P}^J_c$ say, given by

$$\mathbf{P}^J_c = \mathbf{P}^J_{co}\mathbf{P}^J_{oc}, \tag{8}$$

and equilibrium distribution, $\pi^c = (\pi^c_1, \pi^c_2, \ldots, \pi^c_{m_c})^\top$ say, which can be obtained in an analogous fashion to π^o above. Note that the two equilibrium distributions satisfy

$$\pi^c = (\mathbf{P}^J_{oc})^\top \pi^o \quad \text{and} \quad \pi^o = (\mathbf{P}^J_{co})^\top \pi^c.$$

We now give formulae relating commonly used channel properties to parameters of the process $\{(J_k, T_k)\}$.

(*a*) *Sojourn time density functions.* First consider open sojourns. For $i = 1, 2, \ldots, m_o$ let $f_i(t)$ $(t > 0)$ be the pdf of an open sojourn given that the open states are entered via state a_i. Summing over possible entry states for the succeeding closed sojourn, we obtain that

$$f_i(t) = \sum_{j=m_o+1}^{m} f_{ij}(t) \quad (t > 0). \tag{9}$$

Let $f_o(t)$ $(t > 0)$ be the unconditional pdf of an open sojourn. Then

$$f_o(t) = \sum_{i=1}^{m_o} \pi_i^o f_i(t) = (\pi^o)^T \mathbf{f}_{oc}(t)\mathbf{1} \quad (t > 0), \tag{10}$$

where $\mathbf{f}(t)$ has been partitioned as in Eq. 4, and $\mathbf{1}$ is a column vector of ones. (Throughout this paper, $\mathbf{1}$ will denote a column vector of ones whose dimension, in the present case m_c, will be apparent from the context.) Let $f_c(t)$ $(t > 0)$ be the unconditional pdf of a closed sojourn. Similar arguments show that

$$f_c(t) = (\pi^c)^T \mathbf{f}_{co}(t)\mathbf{1} \quad (t > 0).$$

(*b*) *Moments.* For $r = 0, 1, \cdots$ let $M^{(r)} = [M_{ij}^{(r)}]$ be the $m \times m$ matrix given by

$$\mathbf{M}^{(r)} = \int_0^\infty t^r \mathbf{f}(t)\, \mathrm{d}t.$$

Note that $\mathbf{M}^{(0)} = \mathbf{P}^J$. The matrix $\mathbf{M}^{(r)}$ contains information concerning rth moments of open and closed sojourns. First consider open sojourns. For $i = 1, 2, \ldots, m_o$ let $m_i^{(r)} = \int_0^\infty t^r f_i(t)\, \mathrm{d}t$ be the rth moment of the length of open sojourns that start in state a_i. Summing over possible entry states for the succeeding closed sojourn, we obtain that

$$m_i^{(r)} = \sum_{j=m_o+1}^{m} M_{ij}^{(r)}.$$

Let $\mu_o^{(r)}$ be the unconditional rth moment of open sojourns. Then

$$\mu_o^{(r)} = \sum_{i=1}^{m_o} \pi_i^o m_i^{(r)} = (\pi^o)^T \mathbf{M}_{oc}^{(r)}\mathbf{1},$$

where $\mathbf{M}^{(r)}$ has been partitioned as in Eq. 4. Similarly, the unconditional rth moment of closed sojourns is given by

$$\mu_c^{(r)} = (\pi^c)^T \mathbf{M}_{co}^{(r)}\mathbf{1}.$$

Further, we let σ_o^2 and σ_c^2 denote the corresponding variances which can be obtained, for example, using $\sigma_o^2 = \mu_o^{(2)} - [\mu_o^{(1)}]^2$.

Note that the proportion of time that the channel spends in the open class is given by $\mu_o^{(1)}/(\mu_o^{(1)} + \mu_c^{(1)})$.

In practice a closed form expression for $\mathbf{f}(t)$ may not be available, in which case the moment matrices $\mathbf{M}^{(r)}$ can be derived from the Laplace transform $\Phi(\theta)$ using the formulae

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

where $\Phi^{(r)}(\theta)$ is the matrix function obtained by differentiating $\Phi(\theta)$ r times with respect to θ.

(*c*) *Auto- and cross-correlation functions.* Several authors have noted that important information concerning the structure of an ion channel gating mechanism is contained in the open and closed sojourn auto-correlation functions (Fredkin et al., 1985; Colquhoun and Hawkes, 1987; Ball and Sansom, 1988*a*, *b*; Ball and Rice, 1989) and the open-closed and closed-open cross-correlation functions (Ball et al., 1988). In determining auto- and cross-correlation functions for Markov gating mechanisms Ball and Sansom (1988*a*) and Ball et al. (1988) exploited an embedded semi-Markov process. Thus formulae for the correlation functions in our present semi-Markov context can be obtained directly from these papers. For the present discussion of correlation functions only it is convenient to let $S_1, S_2, \cdots$ be the lengths of successive open sojourns and $T_1, T_2, \cdots$ be the lengths of successive closed sojourns. Thus the channel record is $S_1, T_1, S_2, T_2, \ldots$. Then, provided that the channel is in equilibrium,

$$\mathrm{Cov}(S_i, S_{i+k}) = (\pi^o)^T \mathbf{M}_{oc}^{(1)}\mathbf{P}_{co}^J(\mathbf{P}_o^J)^{k-1}\mathbf{M}_{oc}^{(1)}\mathbf{1} - (\mu_o^{(1)})^2 \quad (k = 1, 2, \cdots),$$

$$\mathrm{Cov}(T_i, T_{i+k}) = (\pi^c)^T \mathbf{M}_{co}^{(1)}\mathbf{P}_{oc}^J(\mathbf{P}_c^J)^{k-1}\mathbf{M}_{co}^{(1)}\mathbf{1} - (\mu_c^{(1)})^2 \quad (k = 1, 2, \cdots),$$

$$\mathrm{Cov}(S_i, T_{i+k}) = (\pi^o)^T \mathbf{M}_{oc}^{(1)}(\mathbf{P}_c^J)^k\mathbf{M}_{co}^{(1)}\mathbf{1} - \mu_o^{(1)}\mu_c^{(1)} \quad (k = 0, 1, \cdots),$$

and

$$\mathrm{Cov}(T_i, S_{i+1+k}) = (\pi^c)^T \mathbf{M}_{co}^{(1)}(\mathbf{P}_o^J)^k\mathbf{M}_{oc}^{(1)}\mathbf{1} - \mu_o^{(1)}\mu_c^{(1)} \quad (k = 0, 1, \cdots).$$

If the matrices $\mathbf{P}_o^J$ and $\mathbf{P}_c^J$ are diagonalisable the above formulae admit simple forms. Let $\sigma_1, \sigma_2, \ldots, \sigma_{m_o}$ be the eigenvalues of $\mathbf{P}_o^J$, with corresponding right eigenvectors $\mathbf{b}_1, \mathbf{b}_2, \ldots, \mathbf{b}_{m_o}$. Let $\mathbf{B}$ be the $m_o \times m_o$ matrix whose ith column is $\mathbf{b}_i$ and $\mathbf{C} = \mathbf{B}^{-1}$. Then $\mathbf{P}_o^J$ admits the spectral representation

$$\mathbf{P}_o^J = \sum_{i=1}^{m_o} \sigma_i \mathbf{E}_i \tag{11}$$

where

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

and $\mathbf{c}_i$ is the ith row of $\mathbf{C}$.

Let $m^* = \min(m_o, m_c)$. Then $\mathbf{P}_o^J$ has rank at most m^*, so let $\sigma_1, \sigma_2, \ldots, \sigma_{m^*}$ be the possibly non-zero eigenvalues of $\mathbf{P}_o^J$. One of these eigenvalues, σ_{m^*} say, is one and the remainder have modulus strictly less than one (Cox and Miller, 1965, p. 123). Thus Eq. 11 may be written

$$\mathbf{P}_o^J = \mathbf{E}_{m^*} + \sum_{i=1}^{m^*-1} \sigma_i \mathbf{E}_i, \qquad (12)$$

where the summation is omitted if $m^* = 1$. It follows, see Ball and Sansom (1988*a*) for details, that Cov (S_i, S_{i+k}) can be expressed in the form

$$\text{Cov}\,(S_i, S_{i+k}) = \sum_{j=1}^{m^*-1} \alpha_j \sigma_j^{k-1} \quad (k = 1, 2, \cdots), \qquad (13)$$

where

$$\alpha_j = (\pi^o)^T \mathbf{M}_{oc}^{(1)} \mathbf{P}_{co}^J \mathbf{E}_j \mathbf{M}_{oc}^{(1)} \mathbf{1} \quad (j = 1, 2, \ldots, m^* - 1).$$

If $\mathbf{P}_o^J$ admits the spectral representation in Eq. 12 then $\mathbf{P}_c^J$ admits the spectral representation

$$\mathbf{P}_c^J = \mathbf{F}_{m^*} + \sum_{i=1}^{m^*-1} \sigma_i \mathbf{F}_i$$

(Ball and Rice, 1989), where the matrices $\mathbf{F}_1, \mathbf{F}_2, \ldots, \mathbf{F}_{m^*}$ are derived from $\mathbf{P}_c^J$ in the same way as $\mathbf{E}_1, \mathbf{E}_2, \ldots, \mathbf{E}_{m^*}$ are derived from $\mathbf{P}_o^J$. It follows that

$$\text{Cov}\,(T_i, T_{i+k}) = \sum_{j=1}^{m^*-1} \beta_j \sigma_j^{k-1} \quad (k = 1, 2, \cdots), \qquad (14)$$

where

$$\beta_j = (\pi^c)^T \mathbf{M}_{co}^{(1)} \mathbf{P}_{oc}^J \mathbf{F}_j \mathbf{M}_{co}^{(1)} \mathbf{1} \quad (j = 1, 2, \ldots, m^* - 1).$$

Similar arguments (Ball et al., 1988) show that

$$\text{Cov}\,(S_i, T_{i+k}) = \sum_{j=1}^{m^*-1} \gamma_j \sigma_j^{k} \quad (k = 1, 2, \cdots), \qquad (15)$$

and

$$\text{Cov}\,(T_i, S_{i+1+k}) = \sum_{j=1}^{m^*-1} \delta_j \sigma_j^{k} \quad (k = 1, 2, \cdots), \qquad (16)$$

where

$$\gamma_j = (\pi^o)^T \mathbf{M}_{oc}^{(1)} \mathbf{F}_j \mathbf{M}_{co}^{(1)} \mathbf{1} \quad \text{and} \quad \delta_j = (\pi^c)^T \mathbf{M}_{co}^{(1)} \mathbf{E}_j \mathbf{M}_{oc}^{(1)} \mathbf{1} \quad (j = 1, 2, \ldots, m^* - 1).$$

When $m_o = m_c$, Eqs. 15 and 16 hold for $k = 0$ as well. This does not necessarily follow when $m_o \neq m_c$ as the following example illustrates

$$C_2 \rightleftarrows O_1 \rightleftarrows C_3.$$

Suppose that the gateway semi-Markov process is such that for short (long) sojourns in O_1 the process goes to C_2 (C_3), and that sojourns in C_2 are short compared to sojourns in C_3. Then clearly Cov $(S_1, T_1) > 0$, but $m^* = 1$ so Eq. 15 does not hold with $k = 0$. Note that Cov $(S_1, T_k) = 0$ for $k \geq 2$ so Eq. 15 holds for $k \geq 1$ as indicated.

Correlations corresponding to the above covariances are obtained by dividing the latter by the product of the appropriate standard deviations. For example, we write $\rho_{oo}^{(k)}$ for the correlation (which in the notation of this section is corr (S_i, S_{i+k})) between the length of an open sojourn and that of the kth following open sojourn, $k = 1, 2, \ldots$. Note that the auto-correlations are zero if $m^* = 1$, as are the cross-correlations except perhaps for $k = 0$. The practical importance of the forms in Eqs. 13–16 is that by fitting them to empirical covariance functions a lower bound can in principle be placed on the numbers of open and closed gateway states in the underlying channel gating mechanism (for examples where such inferences have been applied to experimental data see Labarca et al., 1985; Kerry et al., 1987, 1988; and Bates et al., 1990). It is important to note that the forms in Eqs. 13–16 do not require the underlying channel process to be a continuous-time Markov chain and that inferences based on the form of correlation functions remain valid in our more general semi-Markov context.

The underlying semi-Markov process and its associated gateway process

In the above we described the gateway process $\{(J_k, T_k)\}$, which contains all the information concerning observable single channel behavior, and showed how commonly used channel properties can be expressed in terms of its parameters. We now provide a general model for the gating mechanism of a single ion channel and derive the parameters of the corresponding gateway process. Our general model will again be a semi-Markov process. There are two reasons for this choice. First, an underlying single channel process that is semi-Markov necessarily results in the associated gateway process being semi-Markov and secondly, such a framework for the underlying process includes almost all the single channel models currently available in the literature.

Suppose that there are n states that the channel can be in, n_o of which are open and n_c closed. Label the states 1, 2, $\ldots$, n, where 1, 2, $\ldots$, n_o are the open states. For $i = 1, 2, \ldots, n$ let $g_i(t)$ $(t > 0)$ be the pdf of the length of a typical sojourn in state i. On leaving a state, i say, the channel enters state j with probability $p_{ij}(t)$, where t is the length of the sojourn in state i. Thus $\sum_{j \neq i} p_{ij}(t) = 1$ $(t > 0; i = 1, 2, \ldots, n)$. We assume that the sojourn time in a given state is independent of the past history of the channel, and the next state visited depends on the past history only through the current state and its sojourn time. Thus our model for the single channel is completely specified by the functions $g_i(t)$ $(i = 1, 2, \ldots, n)$ and $p_{ij}(t)$ $(i, j = 1, 2, \ldots, n)$, where $p_{ii}(t) \equiv 0$ $(i = 1, 2, \ldots, n)$.

Previously used channel models can be obtained within this general framework as follows. If the functions $p_{ij}(t)$ are independent of t, i.e., $p_{ij}(t) \equiv p_{ij}$ then the above model reduces to that considered by Edeson et al.

(1990). If further the pdfs $g_i(t)$ ($i = 1, 2, \ldots, n$) are all exponential we recover the Markov model so prevalent in channel literature (e.g., Colquhoun and Hawkes, 1982). If $n_o = n_c = 1$ we obtain the alternating renewal process model of Milne et al. (1988) and hence also, by a further specialisation to Weibull sojourn time distributions, the so-called fractal model of Liebovitch et al. (1987).

It is mathematically convenient to use the following equivalent specification of our underlying single channel model. Suppose that the channel changes state at time $t = 0$. Let K_0 be the state the channel is in at time $t = 0$ and $K_1, K_2, \cdots$ be the successive states visited by the channel. Let U_1 be the length of the initial sojourn of the channel in state K_0 and $U_2, U_3, \cdots$ be the lengths of succeeding channel (state as opposed to aggregate) sojourns. Then the single channel process is completely described by the process $\{(K_l, U_l)\}$, with the convention that $U_0 = 0$. Its properties are completely determined by its semi-Markov kernel, i.e., the $n \times n$ matrix function $\mathbf{G}(t) = [G_{ij}(t)]$, defined by

$$G_{ij}(t) = \mathbb{P}(K_l = j \text{ and } U_l \le t | K_{l-1} = i) \quad (t \ge 0; i, j = 1, 2, \ldots, n).$$

Let $\mathbf{g}(t) = [g_{ij}(t)] = \mathbf{G}'(t)$, so $\mathbf{G}(t) = \int_0^t \mathbf{g}(u)\,\mathrm{d}u$. Then, in terms of our earlier notation,

$$g_{ij}(t) = g_i(t)p_{ij}(t) \quad (t > 0; i, j = 1, 2, \ldots, n).$$

Conversely, the functions $g_i(t)$ and $p_{ij}(t)$ can be expressed in terms of $g_{ij}(t)$ as follows:

$$g_i(t) = \sum_{j=1}^{n} g_{ij}(t) \quad (t > 0; i = 1, 2, \ldots, n)$$

and

$$p_{ij}(t) = g_{ij}(t)/g_i(t) \quad (t > 0; i, j = 1, 2, \ldots, n).$$

Thus the two specifications are equivalent. Note that the densities $g_{ij}(t)$ and distribution functions $G_{ij}(t)$ will usually be defective, in the sense that

$$\int_0^\infty g_{ij}(t)\,\mathrm{d}t = G_{ij}(\infty) = \mathbb{P}(K_l = j | K_{l-1} = i)$$

is less than one.

Now suppose that there are m_o open and m_c closed gateway states, that the open gateway states are $1, 2, \ldots, m_o$ and the closed gateway states $n_o + 1, n_o + 2, \ldots, n_o + m_c$. To connect with previous notation $a_i = i$ ($i = 1, 2, \ldots, m_o$) and $a_{m_o+j} = n_o + j$ ($j = 1, 2, \ldots, m_c$). The gateway semi-Markov process $\{(J_k, T_k)\}$ has already been defined, so all that is required to determine the observed channel properties are expressions for the parameters of $\{(J_k, T_k)\}$ in terms of those of the underlying process $\{(K_l, U_l)\}$.

(a) Semi-Markov Kernel $\boldsymbol{F}(t)$. Let $\Psi(\theta) = [\Psi_{ij}(\theta)]$ be the $n \times n$ matrix function defined by

$$\Psi(\theta) = \int_0^\infty \exp(-\theta t)\mathbf{g}(t)\,\mathrm{d}t \quad (\theta \ge 0),$$

i.e., $\Psi(\theta)$ is the Laplace transform of $\mathbf{g}(t)$. Partition $\Psi(\theta)$ into

$$\Psi(\theta) = \begin{bmatrix} \Psi_{oo}(\theta) & \Psi_{oc}(\theta) \\ \Psi_{co}(\theta) & \Psi_{cc}(\theta) \end{bmatrix}, \tag{17}$$

where $\Psi_{oo}(\theta)$ corresponds to transitions that remain within the open states and $\Psi_{oc}(\theta)$ to transitions from the open states to the closed states, etc. Let

$$\Psi_0(\theta) = \begin{bmatrix} \Psi_{oo}(\theta) & \mathbf{0} \\ \mathbf{0} & \Psi_{cc}(\theta) \end{bmatrix},$$

$$\Psi_1(\theta) = \begin{bmatrix} \mathbf{0} & \Psi_{oc}(\theta) \\ \Psi_{co}(\theta) & \mathbf{0} \end{bmatrix},$$

and (18)

$$\mathbf{L} = \begin{bmatrix} \mathbf{I} & \mathbf{0} & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{I} & \mathbf{0} \end{bmatrix} \begin{matrix} m_o \\ m_c, \end{matrix}$$
$$\quad m_o \quad n_o - m_o \quad m_c \quad n_c - m_c$$

where the dimensions of the partitioned form of $\mathbf{L}$ are shown. Then it follows from Theorem 3.1 of Ball et al. (1991) that

$$\Phi(\theta) = \mathbf{L}(\mathbf{I} - \Psi_0(\theta))^{-1}\Psi_1(\theta)\mathbf{L}^{\mathrm{T}} \quad (\theta \ge 0). \tag{19}$$

Equation 19 can be explained briefly as follows. Note that Eq. 19 can be expressed as

$$\Phi(\theta) = \mathbf{L}\left(\sum_{n=0}^{\infty} \Psi_0(\theta)^n \Psi_1(\theta)\right)\mathbf{L}^{\mathrm{T}} \quad (\theta \ge 0),$$

which on expansion in partitioned form yields

$$\Phi(\theta) = \mathbf{L}\begin{bmatrix} \mathbf{0} & \sum_{n=0}^{\infty} \Psi_{oo}(\theta)^n \Psi_{oc}(\theta) \\ \sum_{n=0}^{\infty} \Psi_{cc}(\theta)^n \Psi_{co}(\theta) & \mathbf{0} \end{bmatrix}\mathbf{L}^{\mathrm{T}} \quad (\theta \ge 0).$$

An open sojourn consists of a number, n say, of sojourns in open states for which the next state visited is also open ($n = 0, 1, \cdots$) followed by a sojourn in an open state for which the next state visited is a closed state. It is easily verified that the corresponding contribution to $\Phi(\theta)$ is $\Psi_{oo}(\theta)^n\Psi_{oc}(\theta)$. The matrices $\mathbf{L}$ and $\mathbf{L}^{\mathrm{T}}$ have the effect of taking the action down from the full state space to the subset of gateway states.

To obtain $\mathbf{f}(t)$, and hence $\mathbf{F}(t)$, we need to invert the Laplace transform $\Phi(\theta)$. For some classes of model, e.g., Markov and alternating renewal models, this can be done analytically to yield closed form formulae for $\mathbf{f}(t)$ and $\mathbf{F}(t)$. For other models numerical inversion of the Laplace transform $\Phi(\theta)$, or an alternative method of determining $\mathbf{f}(t)$, will be required. When the underlying gating mechanism has no cycles within the open or closed states, and $p_{ij}(t)$ is independent of t for all i and j, the recursive methods of Edeson et al. (1990) may facilitate analytic inversion of $\Phi(\theta)$. However, many important channel properties, including moments and correlation functions, can be derived without inversion of $\Phi(\theta)$.

(b) Transition matrices of open and closed entry processes. First let $\mathbf{P}^K = [P^K_{ij}]$ be the transition matrix of $\{K_l\}$, i.e.,

$$P^K_{ij} = \mathbb{P}(K_l = j | K_{l-1} = i) \quad (i, j = 1, 2, \ldots, n).$$

Then $\mathbf{P}^K = \Psi(0)$. Partition $\mathbf{P}^K$ as in Eq. 17 and define matrices $\mathbf{P}^K_0$ and $\mathbf{P}^K_1$ in a similar fashion to Eq. 18. Then, since $\mathbf{P}^J = \Phi(0)$, it follows from Eq. 19 that

$$\mathbf{P}^J = \mathbf{L}(\mathbf{I} - \mathbf{P}^K_0)^{-1}\mathbf{P}^K_1\mathbf{L}^\top.$$

Recalling Eqs. 5 and 8, it follows after a little algebra that

$$\begin{bmatrix} \mathbf{P}^J_o & \mathbf{0} \\ \mathbf{0} & \mathbf{P}^J_c \end{bmatrix} = \mathbf{L}[(\mathbf{I} - \mathbf{P}^K_0)^{-1}\mathbf{P}^K_1]^2\mathbf{L}^\top, \qquad (20)$$

so the transition matrices of the open and closed entry processes are determined.

(c) Equilibrium distributions of open and closed entry processes. The equilibrium distributions π^o and π^c can be derived from the transition matrices $\mathbf{P}^J_o$ and $\mathbf{P}^J_c$ as described earlier. Alternatively, if the Markov chain $\{K_l\}$, which records the successive states of the channel, has equilibrium distribution $\pi^K = (\pi^K_1, \pi^K_2, \ldots, \pi^K_n)^\top$, then it is shown in Ball et al. (1991) that

$$\pi^o_i = \alpha_o \sum_{l=n_o+1}^{n} \pi^K_l p^K_{li} \quad (i = 1, 2, \ldots, m_o) \qquad (21)$$

$$\pi^c_j = \alpha_c \sum_{l=1}^{n_o} \pi^K_l p^K_{l,n_o+j} \quad (j = 1, 2, \ldots, m_c), \qquad (22)$$

where α_o and α_c are normalizing constants, chosen so that the respective distributions sum to one.

(d) Moments. For $r = 0, 1, \cdots$ let $\hat{\mathbf{M}}^{(r)} = [\hat{M}^{(r)}_{ij}]$ be the $n \times n$ matrix defined by

$$\hat{\mathbf{M}}^{(r)} = \int_0^\infty t^r \mathbf{g}(t)\, dt = (-1)^r \Psi^{(r)}(0).$$

(The notation adopted here differs slightly from that in Ball et al. (1991).) Partition $\hat{\mathbf{M}}^{(r)}$ as in Eq. 17 and define matrices $\hat{\mathbf{M}}^{(r)}_0$ and $\hat{\mathbf{M}}^{(r)}_1$ in a similar fashion to Eq. 18. Then, by appropriate differentiation of Eq. 19, it follows that

$$\mathbf{M}^{(r)} = \mathbf{L}\mathbf{A}^{(r)}\mathbf{L}^\top \qquad (23)$$

where the $n \times n$ matrices $\mathbf{A}^{(r)}$ $(r = 0, 1, \cdots)$ are defined by

$$\mathbf{A}^{(r)} = (\mathbf{I} - \hat{\mathbf{M}}^{(0)}_0)^{-1}\left\{\hat{\mathbf{M}}^{(r)}_1 + \sum_{k=0}^{r-1} \binom{r}{k} \hat{\mathbf{M}}^{(r-k)}_0 \mathbf{A}^{(k)}\right\} \quad (r = 0, 1, \cdots), \qquad (24)$$

and the summation is omitted if $r = 0$.

Note that $\hat{\mathbf{M}}^{(0)} = \mathbf{P}^K$ and $\mathbf{M}^{(0)} = \mathbf{P}^J$.

Time interval omission

We now show how to derive properties of the observed channel process when brief events are not able to be detected. Recall the gateway process $\{(\tilde{J}_k, \tilde{T}_k)\}$ which arises when there is time interval omission. This is a semi-Markov process and is analogous to the process $\{(J_k, T_k)\}$ which arises in the absence of time interval omission. Thus observed channel properties incorporating time interval omission follow from results given earlier for $\{(J_k, T_k)\}$ once the parameters of $\{(\tilde{J}_k, \tilde{T}_k)\}$ have been derived. For these parameters we use symbols as in the absence of time interval omission, but embellished by tildes. For ease of exposition we consider the case when the detection limits for both open and closed sojourns are constant and equal to τ. The cases of unequal and variable detection limits are treated in Ball et al. (1991), where proofs may be found. Here we just state the results, but first give some more notation.

Let

$$\Phi_U(\theta) = \int_0^\tau \exp(-\theta t)\mathbf{f}(t)\, dt,$$

$$\Phi_D(\theta) = \int_\tau^\infty \exp(-\theta t)\mathbf{f}(t)\, dt \quad (\theta \geq 0),$$

where U and D correspond to undetected and detected sojourns, respectively. Thus $\Phi(\theta) = \Phi_U(\theta) + \Phi_D(\theta)$ $(\theta \geq 0)$. Let $\mathbf{D} = [d_{ij}]$ be the $m \times m$ diagonal matrix, with diagonal elements given by

$$d_{ii} = 1 - \sum_{j=1}^{m} F_{ij}(\tau) \quad (i = 1, 2, \cdots m).$$

Thus d_{ii} is the probability that a sojourn commencing in state i is detected. Let $\pi^E = (\pi^o_1, \pi^o_2, \ldots, \pi^o_{m_o}, \pi^c_1, \pi^c_2, \ldots, \pi^c_{m_c})^\top$. For $r = 0, 1, \cdots$ let

$$\mathbf{M}^{(r)}_U = \int_0^\tau t^r \mathbf{f}(t)\, dt = (-1)^r \Phi^{(r)}_U(0) \qquad (25)$$

and $\mathbf{M}^{(r)}_D = \mathbf{M}^{(r)} - \mathbf{M}^{(r)}_U$. Note that $\mathbf{M}^{(0)}_U = \mathbf{F}(\tau)$.

(a) Semi-Markov kernel. Let $\tilde{\mathbf{F}}(t) = [\tilde{F}_{ij}(t)]$ be the $m \times m$ matrix function defined by

$$\tilde{F}_{ij}(t) = \mathbb{P}(\tilde{J}_k = a_j \text{ and } \tilde{T}_k \leq t | \tilde{J}_{k-1} = a_i) \quad (t \geq 0; i, j = 1, 2, \ldots, m),$$

$\tilde{\mathbf{f}}(t) = \tilde{\mathbf{F}}'(t)$ $(t > 0)$ and $\tilde{\Phi}(\theta) = \int_0^\infty \exp(-\theta t)\tilde{\mathbf{f}}(t)\, dt$ $(\theta \geq 0)$. Then it follows from Theorem 4.2 of Ball et al. (1991) that

$$\tilde{\Phi}(\theta) = \mathbf{D}^{-1}\Phi_D(\theta)\{\mathbf{I} - \Phi_U(\theta)\Phi(\theta)\}^{-1}\mathbf{D} \quad (\theta \geq 0). \quad (26)$$

Eq. 26 can be explained briefly as follows. Note that Eq. 26 can be expressed as

$$\tilde{\Phi}(\theta) = \sum_{n=0}^{\infty} \mathbf{D}^{-1}\Phi_D(\theta)(\Phi_U(\theta)\Phi(\theta))^n\mathbf{D} \quad (\theta \geq 0).$$

An observed open sojourn consists of an initial sojourn of length at least τ in the open states, followed by a number, n say, of undetected closed sojourns and arbitrary open sojourns, and terminates with a sojourn of length at least τ in the closed states. It is easily verified that the corresponding contributions to $\tilde{\Phi}(\theta)$ are appropriate partitions of $\mathbf{D}^{-1}\Phi_D(\theta)$, $(\Phi_U(\theta)\Phi(\theta))^n$ and $\mathbf{D}$, respectively.

In practice, it is generally not possible to invert analytically the Laplace transform $\tilde{\Phi}(\theta)$ to obtain a closed form expression for $\tilde{\mathbf{f}}(t)$ (though see Ball et al., 1993), so numerical inversion or alternative techniques will be needed if, for example, observed sojourn time pdfs are required. However, for Markov models Hawkes et al. (1990) derive a closed form recursive formula for $\tilde{\mathbf{f}}(t)$ by term-wise inversion of an infinite series expansion for the Laplace transform.

(b) Transition matrices of observed open and closed entry processes. The transition matrix, $\tilde{\mathbf{P}}^J$ say, of the Markov chain $\{\tilde{J}_k\}$ is given by

$$\tilde{\mathbf{P}}^J = \tilde{\Phi}(0) = \mathbf{D}^{-1}\mathbf{M}_D^{(0)}(\mathbf{I} - \mathbf{M}_U^{(0)}\mathbf{P}^J)^{-1}\mathbf{D}. \quad (27)$$

The transition matrices, $\tilde{\mathbf{P}}_o^J$ and $\tilde{\mathbf{P}}_c^J$ say, of the observed open and closed entry processes, $\{\tilde{J}_{2k}\}$ and $\{\tilde{J}_{2k+1}\}$, are given by

$$\begin{bmatrix} \tilde{\mathbf{P}}_o^J & \mathbf{0} \\ \mathbf{0} & \tilde{\mathbf{P}}_c^J \end{bmatrix} = (\tilde{\mathbf{P}}^J)^2. \quad (28)$$

(c) Equilibrium behavior. Let $\tilde{\pi}^o = (\tilde{\pi}_1^o, \tilde{\pi}_2^o, \ldots, \tilde{\pi}_{m_o}^o)^T$ and $\tilde{\pi}^c = (\tilde{\pi}_1^c, \tilde{\pi}_2^c, \ldots, \tilde{\pi}_{m_c}^c)$ be, respectively, the equilibrium distributions of the time interval omission open and closed entry processes $\{\tilde{J}_{2k}\}$ and $\{\tilde{J}_{2k+1}\}$. The equilibrium distributions $\tilde{\pi}^o$ and $\tilde{\pi}^c$ can be derived from $\tilde{\mathbf{P}}^J$ in an analogous fashion to that described earlier for the case of no time interval omission. Alternatively they are given by

$$\tilde{\pi}_i^o = \tilde{\alpha}_o d_{ii}[\{\mathbf{I} + \mathbf{F}(\tau)^T\}^{-1}\pi^E]_i \quad (i = 1, 2, \ldots, m_o), \quad (29)$$

$$\tilde{\pi}_j^c = \tilde{\alpha}_c d_{j+m_o, j+m_o}[\{\mathbf{I} + \mathbf{F}(\tau)^T\}^{-1}\pi^E\}_{j+m_o} \quad (j = 1, 2, \ldots, m_c), \quad (30)$$

where $\tilde{\alpha}_o$ and $\tilde{\alpha}_c$ are normalizing constants.

(d) Moments. For $r = 0, 1, \cdots$ let $\tilde{\mathbf{M}}^{(r)}$ be the $m \times m$ matrix given by

$$\tilde{\mathbf{M}}^{(r)} = \int_0^\infty t^r\tilde{\mathbf{f}}(t)\,dt = (-1)^r\tilde{\Phi}^{(r)}(0).$$

Thus $\tilde{\mathbf{M}}^{(r)}$ can be obtained by appropriate differentiation of Eq. 26. We obtain that

$$\tilde{\mathbf{M}}^{(0)} = \mathbf{D}^{-1}\mathbf{M}_D^{(0)}(\mathbf{I} - \mathbf{M}_U^{(0)}\mathbf{M}^{(0)})^{-1}\mathbf{D},$$
$$\tilde{\mathbf{M}}^{(1)} = \{\tilde{\mathbf{M}}^{(0)}\mathbf{D}^{-1}(\mathbf{M}_U^{(1)}\mathbf{M}^{(0)} + \mathbf{M}_U^{(0)}\mathbf{M}^{(1)}) + \mathbf{D}^{-1}\mathbf{M}_D^{(1)}\} \times (\mathbf{I} - \mathbf{M}_U^{(0)}\mathbf{M}^{(0)})^{-1}\mathbf{D},$$
$$\tilde{\mathbf{M}}^{(2)} = \{2\tilde{\mathbf{M}}^{(1)}\mathbf{D}^{-1}(\mathbf{M}_U^{(1)}\mathbf{M}^{(0)} + \mathbf{M}_U^{(0)}\mathbf{M}^{(1)}) + \tilde{\mathbf{M}}^{(0)}\mathbf{D}^{-1}(\mathbf{M}_U^{(2)}\mathbf{M}^{(0)} + 2\mathbf{M}_U^{(1)}\mathbf{M}^{(1)} + \mathbf{M}_U^{(0)}\mathbf{M}^{(2)}) + \mathbf{D}^{-1}\mathbf{M}_D^{(2)}\}(\mathbf{I} - \mathbf{M}_U^{(0)}\mathbf{M}^{(0)})^{-1}\mathbf{D}. \quad (31)$$

Unconditional rth moments of observed open and closed sojourns are denoted by $\tilde{\mu}_o^{(r)}$ and $\tilde{\mu}_c^{(r)}$, respectively. Further, we let $\tilde{\sigma}_o^2$ and $\tilde{\sigma}_c^2$ denote the corresponding variances. These, together with auto- and cross-correlations, can be obtained in a manner analogous to the case without time interval omission. Note that the forms of Eqs. 13–16 still pertain, so inferences based on correlation functions are not affected by time interval omission.

Markov case

We now consider the special case when the underlying single channel process $\{X(t)\}$ is a continuous-time Markov chain, and provide formulae which enable our general framework to be used to determine model observed channel properties both with and without time interval omission. Derivations of our formulae can be found in Ball et al. (1991). The formulae not involving time interval omission can also be deduced from, for example, Colquhoun and Hawkes (1977). First we need to describe the model and some further notation.

Suppose there are n states labelled precisely as before. For $i \neq j$ let q_{ij} be the transition rate of the channel from state i to state j. Let $\mathbf{Q}$ be the $n \times n$ matrix with off-diagonal elements q_{ij} and diagonal elements $q_{ii} = -\sum_{i \neq j} q_{ij}$. Partition $\mathbf{Q}$ into

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

and let

$$\mathbf{Q}_0 = \begin{bmatrix} \mathbf{Q}_{oo} & \mathbf{0} \\ \mathbf{0} & \mathbf{Q}_{cc} \end{bmatrix}, \quad \mathbf{Q}_1 = \begin{bmatrix} \mathbf{0} & \mathbf{Q}_{oc} \\ \mathbf{Q}_{co} & \mathbf{0} \end{bmatrix}.$$

For $i = 1, 2, \ldots, n$ let π_i be the equilibrium probability that the channel is in state i, and $\pi = (\pi_1, \pi_2, \ldots, \pi_n)^T$. Then π is determined by $\pi^T\mathbf{Q} = 0$ and $\pi^T\mathbf{1} = 1$. Note that π will usually be different from π^K described earlier, since the latter takes no account of the sojourn times of the channel in the various states.

The continuous-time Markov chain $\{X(t)\}$ is also, of course, a semi-Markov process. Although it is not re-

TABLE 1 Properties of gateway process, with and without time interval omission, when $\{X(t)\}$ is a continuous-time Markov chain

Property of gateway process	Without time interval omission $\{(J_k, T_k)\}$	With time interval omission $\{(\tilde{J}_k, \tilde{T}_k)\}$
Semi-Markov kernel $\mathbf{F}(t)$	$-\mathbf{L}\mathbf{Q}_0^{-1}[\mathbf{I} - \exp(\mathbf{Q}_0 t)]\mathbf{Q}_1\mathbf{L}^{\mathsf{T}}$	Via numerical inversion of $\tilde{\Phi}(\theta)$
density $\mathbf{f}(t)$	$\mathbf{L}\exp(\mathbf{Q}_0 t)\mathbf{Q}_1\mathbf{L}^{\mathsf{T}}$	Numerical inversion of $\tilde{\Phi}(\theta)$
Laplace transform $\Phi(\theta)$	$\mathbf{L}(\theta\mathbf{I} - \mathbf{Q}_0)^{-1}\mathbf{Q}_1\mathbf{L}^{\mathsf{T}}$	From Eq. 26 with $\mathbf{D} = \mathbf{L}\,\mathrm{diag}\,\{\mathbf{A}(\tau)\mathbf{1}\}\mathbf{L}^{\mathsf{T}}$, $\Phi_{\mathrm{D}}(\theta) = \exp(-\theta\tau)\mathbf{L}(\theta\mathbf{I} - \mathbf{Q}_0)^{-1}\mathbf{A}(\tau)\mathbf{Q}_1\mathbf{L}^{\mathsf{T}}$ and $\Phi_{\mathrm{U}}(\theta) = \Phi(\theta) - \Phi_{\mathrm{D}}(\theta)$
Transition matrices of open and closed entry processes $\begin{bmatrix}\mathbf{P}_{\mathrm{o}}^{J} & \mathbf{0}\\ \mathbf{0} & \mathbf{P}_{\mathrm{c}}^{J}\end{bmatrix}$	$\mathbf{L}(\mathbf{Q}_0\mathbf{Q}_1)^2\mathbf{L}^{\mathsf{T}}$	$\mathbf{L}\mathbf{D}^{-1}[\mathbf{Q}_0^{-1}\mathbf{A}(\tau)\mathbf{Q}_1\{\mathbf{I} - \mathbf{Q}_0^{-1}(\mathbf{I} - \mathbf{A}(\tau))\mathbf{Q}_1\mathbf{Q}_0^{-1}\mathbf{Q}_1\}^{-1}]^2\mathbf{D}\mathbf{L}^{\mathsf{T}}$
Equilibrium distributions, π^{o} and π^{c} of open and closed entry process	$\pi_i^{\mathrm{o}} = \alpha_{\mathrm{o}} \sum_{l=n_{\mathrm{o}}+1}^{n} \pi_l q_{li}$ $(i = 1, 2, \ldots, m_{\mathrm{o}})$ $\pi_j^{\mathrm{c}} = \alpha_{\mathrm{c}} \sum_{l=1}^{n_{\mathrm{o}}} \pi_l q_{l,n_{\mathrm{o}}+j}$ $(j = 1, 2, \ldots, m_{\mathrm{c}})$	From Eqs. 29 and 30 with $\mathbf{F}(\tau) = -\mathbf{L}\mathbf{Q}_0^{-1}[\mathbf{I} - \mathbf{A}(\tau)]\mathbf{Q}_1\mathbf{L}^{\mathsf{T}}$ and $\mathbf{D}$ as above
Moment matrices	$\mathbf{M}^{(r)} = (-1)^{r+1} r!\,\mathbf{L}\mathbf{Q}_0^{-(r+1)}\mathbf{Q}_1\mathbf{L}^{\mathsf{T}}$ $(r = 0, 1, \ldots)$	$\tilde{\mathbf{M}}^{(r)}$ $(r = 0, 1, 2)$ from Eq. 31 with $\mathbf{M}_{\mathrm{D}}^{(0)} = -\mathbf{L}\mathbf{Q}_0^{-1}\mathbf{A}(\tau)\mathbf{Q}_1\mathbf{L}^{\mathsf{T}}$, $\mathbf{M}_{\mathrm{D}}^{(1)} = \mathbf{L}(\mathbf{Q}_0^{-2} - \tau\mathbf{Q}_0^{-1})\mathbf{A}(\tau)\mathbf{Q}_1\mathbf{L}^{\mathsf{T}}$, $\mathbf{M}_{\mathrm{D}}^{(2)} = \mathbf{L}(2\mathbf{Q}_0^{-3} - 2\tau\mathbf{Q}_0^{-2} + \tau^2\mathbf{Q}_0^{-1})\mathbf{A}(\tau)\mathbf{Q}_1\mathbf{L}^{\mathsf{T}}$, $\mathbf{M}_{\mathrm{U}}^{(r)} = \mathbf{M}^{(r)} - \mathbf{M}_{\mathrm{D}}^{(r)}$ $(r = 0, 1, 2)$ and $\mathbf{D}$ as above

The parameters given in the left-hand column are for the case of no time interval omission. Those with time interval omission are found by replacing, for example, $\mathbf{F}(t)$ with $\tilde{\mathbf{F}}(t)$. For details of parameters and notation see text. Throughout this table $\mathbf{A}(\tau) = \exp(\mathbf{Q}_0\tau) = \sum_{i=0}^{\infty} \tau^i\mathbf{Q}_0^i/i!$ is the usual matrix exponential (e.g., Bellman, 1960, p. 165), and diag { } denotes the diagonal matrix whose entries are the elements of the vector specified in the braces.

quired explicitly here, to aid connection with our earlier theory note that the semi-Markov kernel of $\{X(t)\}$ is given elementwise by

$$G_{ij}(t) = \begin{cases} (-q_{ij}/q_{ii})(1 - e^{q_{ii}t}) & \text{if } i \neq j, \\ 0 & \text{if } i = j. \end{cases}$$

The properties of the gateway processes, $\{(J_k, T_k)\}$ without time interval omission and $\{(\tilde{J}_k, \tilde{T}_k)\}$ with time interval omission, required to model channel properties are listed in Table 1 above.

COMPUTATIONAL CONSIDERATIONS

The semi-Markov framework readily lends itself to structured and efficient numerical computation of model channel properties. The hub of such calculations is the gateway semi-Markov process and its relationship to observed channel properties, as indicated in Table 2.

TABLE 2 Parameters of $\{(J_k, T_k)\}$ required to compute single channel properties when there is no time interval omission

Single channel property	Required parameters of gateway process $\{(J_k, T_k)\}$
Sojourn time pdfs	$\mathbf{f}(t)$, π^0, π^{c}
Moments $(r = 0, 1, 2)$	$\mathbf{M}^{(0)}$ $(=\mathbf{P}^J)$, $\mathbf{M}^{(1)}$, $\mathbf{M}^{(2)}$, π^{o}, π^{c}
Correlations	$\mathbf{M}^{(0)}$, $\mathbf{M}^{(1)}$, $\mathbf{M}^{(2)}$, π^{o}, π^{c}

When there is time interval omission, the required parameters of $\{(\tilde{J}_k, \tilde{T}_k)\}$ are obtained by replacing $\mathbf{f}(t)$ by $\tilde{\mathbf{f}}(t)$ etc.

When there is no time interval omission the parameters in the right-hand column of Table 2 can be calculated from parameters of the underlying single channel process as follows:

- $\mathbf{f}(t)$ by numerical inversion of its Laplace transform $\Phi(\theta)$, given by Eq. 19;
- $\mathbf{M}^{(r)}$ from moments of underlying process using Eqs. 23 and 24;
- π^{o}, π^{c} either directly from the transition matrix and equilibrium distribution of the underlying entry process $\{J_k\}$ using Eqs. 21 and 22, or from $\mathbf{P}_{\mathrm{o}}^{J}$ and $\mathbf{P}_{\mathrm{c}}^{J}$ (given by Eq. 20) using the method described by Eqs. 6 and 7.

When there is time interval omission the parameters can be calculated as follows:

- $\tilde{\mathbf{f}}(t)$ by numerical inversion of its Laplace transform $\tilde{\Phi}(\theta)$, given by Eq. 26 (for calculation of $\mathbf{D}$, $\Phi_{\mathrm{D}}(\theta)$, $\Phi_{\mathrm{U}}(\theta)$, see sequel);
- $\tilde{\mathbf{M}}^{(r)}$ by direct substitution in Eq. 31 (for calculation of $\mathbf{D}$, $\mathbf{M}_{\mathrm{D}}^{(r)}$, $\mathbf{M}_{\mathrm{U}}^{(r)}$, see sequel);
- $\tilde{\pi}^{\mathrm{o}}$, $\tilde{\pi}^{\mathrm{c}}$ either directly from Eqs. 29 and 30, or from $\tilde{\mathbf{P}}_{\mathrm{o}}^{J}$ and $\tilde{\mathbf{P}}_{\mathrm{c}}^{J}$ (given by Eqs. 27 and 28) using the method described by Eqs. 6 and 7.

In the Markov case explicit formulae for $\mathbf{D}$, $\Phi_{\mathrm{D}}(\theta)$, $\Phi_{\mathrm{U}}(\theta)$, $\mathbf{M}_{\mathrm{D}}^{(r)}$, and $\mathbf{M}_{\mathrm{U}}^{(r)}$ are given in Table 1. All the computations are straightforward except perhaps the matrix exponential $\exp(\mathbf{Q}_0\tau)$. If the underlying process $\{X(t)\}$ is time-reversible (i.e., $\pi_i q_{ij} = \pi_j q_{ji}$ for all i and j) then $\mathbf{Q}_0$ is necessarily diagonalisable, which facilitates efficient computation of $\exp(\mathbf{Q}_0\tau)$; see Ball and Sansom (1988b) for details. If $\{X(t)\}$ is not time-reversible then the above approach can be used only if $\mathbf{Q}_0$ is diagonalisable. Otherwise an alternative method of computing $\exp(\mathbf{Q}_0\tau)$ is required. Moler and Van Loan (1978) contains several such methods. In many practical situations τ will be small, so truncation of the series $\sum_{i=0}^{\infty} \tau^i \mathbf{Q}_0^i / i!$ should be fairly accurate, though problems can arise (Moler and Van Loan, 1978; see also Horn and Lange, 1983).

In the non-Markov case closed form expressions for $\mathbf{D}$, $\Phi_D(\theta)$, $\Phi_U(\theta)$, $\mathbf{M}_D^{(r)}$, and $\mathbf{M}_U^{(r)}$ are usually unavailable, and these parameters have to be determined numerically. Now $\mathbf{D} = \text{diag}\,\{\mathbf{M}_D^{(0)}\}$, $\Phi_D(\theta) = \Phi(\theta) - \Phi_U(\theta)$, and $\mathbf{M}_D^{(r)} = \mathbf{M}^{(r)} - \mathbf{M}_U^{(r)}$, so it is sufficient to determine $\Phi_U(\theta)$ and $\mathbf{M}_U^{(r)}$. Further, $\Phi_U(\theta)$ is required only to calculate sojourn time pdfs incorporating time interval omission. An obvious approach to determining $\Phi_U(\theta)$ and $\mathbf{M}_U^{(r)}$ is to use numerical inversion of Laplace transforms, viewing each as a function of τ for fixed θ and r, respectively. However, preliminary investigations using NAG subroutine C06LAF suggest that sufficiently accurate results may be difficult to obtain. Moreover, if sojourn time pdfs incorporating time interval omission are required, numerical inversion of the Laplace transform $\tilde{\Phi}(\theta)$ is also needed.

An alternative approach to determining $\mathbf{M}_U^{(r)}$ is outlined in the appendix. This method, which employs integral equations, is both efficient and accurate (see next section). Its extension to calculating $\tilde{\mathbf{f}}(t)$, and hence sojourn time pdfs incorporating time interval omission, is described elsewhere.

NUMERICAL EXAMPLES

As described above our results and methods are rather general and are applicable to most of the kinetic schemes discussed in the single channel literature. Many properties of the aggregated process, with and without time interval omission, may be computed; here we concentrate on equilibrium behavior, moments and correlations. Particular reference is given to the five-state agonist-gated Markov model (Eq. 1) as the detection limit τ and the agonist concentration a vary. Other Markov models such as the ten-state glutamate receptor-channel model in Ball and Sansom (1987, p. 351) and the seven-state chloride channel model (Eq. 2) of Blatz and Magleby (1989) have also been examined and show results of a similar nature, but are not given here. In the former case the results of that paper are identical in appropriate places to ours, otherwise showing only very minor differences due to the slightly different methods of modelling time interval omission.

We have written Fortran programs with extensive use of the NAG library routines, for reversible Markov models and for semi-Markov models (and partially for irreversible Markov models). A program for the alternating renewal model of Milne et al. (1988) may be regarded as a special case of that for the semi-Markov model. These programs provide information on moments, correlations, cross-correlations and equilibrium behavior of the processes without and with time interval omission; work is continuing on numerical evaluation of sojourn time density functions with time interval omission and on irreversible Markov models. For a Markov model the number of open and closed states, the number of open and closed gateway states, the elements of the $\mathbf{Q}$ matrix and detection limit must be specified. For the non-Markov situation properties (moments, distribution function) of individual state sojourn times and the transition probability matrix must be specified instead of the $\mathbf{Q}$ matrix; furthermore the number of points used in numerically solving the integral equation Eq. A3 must be given. As they are numerically simpler, Markov models have been used as a control on the numerical procedures required in the more general semi-Markov case. For Markov models the two programs naturally give identical numerical results without time interval omission, and so far agree to several significant figures with time interval omission. For a Markov model such as Eq. 32 below, processing time for a run is of the order of 0.5 s on a microVax (1 s for the seven-state model Eq. 2). For non-Markov models most CPU time is taken with solving integral equations; representative runs with a five-state non-Markov model take of the order of 10–30 s.

We now return to the five-state model of Colquhoun and Hawkes (1982), §4, with their basic parameter values but with the possibility of varying agonist concentration:

$$\begin{array}{ccccc} C & \rightleftarrows & CA_1 & \rightleftarrows & CA_2 \\ & & \upharpoonleft\downharpoonright & & \upharpoonleft\downharpoonright \\ & & OA_1 & \rightleftarrows & OA_2 \end{array}$$

Throughout time is in milliseconds (ms) and concentration in micromolar (μM). The transition rate matrix $\mathbf{Q}$ corresponding to Eq. 4.2 of Colquhoun and Hawkes (1982) is, for agonist concentration a,

$$\mathbf{Q} = \begin{bmatrix} -3 - 0.5a & 0.5a & 0 & 3 & 0 \\ 0.000667 & -0.500667 & 0.5 & 0 & 0 \\ 0 & 15 & -19 & 4 & 0 \\ 0.015 & 0 & 0.5a & -2.015 - 0.5a & 2 \\ 0 & 0 & 0 & 0.1a & -0.1a \end{bmatrix}. \tag{32}$$

It is important to note that q_{21} needs to be set to 0.002/3 etc. in the program, otherwise errors will occur in subroutines which assume time reversibility. A selection of results is given in Table 3 and Fig. 3 for a range of values of τ and a. The actual program provides considerably more information than given here, e.g., the probability that a particular sojourn is undetected $(\mathbf{M}_U^{(0)})_{ij}$, the various equilibrium distributions (π^K, π^o, π^c, $\tilde{\pi}^o$, $\tilde{\pi}^c$), and further correlations and cross-correlations.

In the underlying process, transitions from 2 to 1 are very rare while those from 1 to 2 increase proportionally

TABLE 3 Properties of observed sojourn times

a	τ	$\tilde{p}_o^U$	$\tilde{p}_c^U$	$\tilde{\mu}_o^{(1)}$ ($\tilde{\sigma}_o$)	$\tilde{\mu}_c^{(1)}$ ($\tilde{\sigma}_c$)	$\tilde{\pi}_2^o$	$\tilde{\pi}_o(a)$	$\tilde{\rho}_{oo}^{(1)}$ / $\tilde{\rho}_{cc}^{(1)}$	$\tilde{\rho}_{oc}^{(0)}$
μM	*ms*			*ms*	*ms*				
0.1	0	0	0	1.877	992.7	0.926	0.0019	0.0103	−0.0648
				(1.974)	(2556.8)			0.0875	
0.1	0.05	0.0332	0.4485	3.523	1855.1	0.881	0.0019	0.0126	−0.0647
				(3.830)	(3349.6)			0.0472	
0.1	0.10	0.0643	0.6224	5.362	2806.5	0.845	0.0019	0.0091	−0.0453
				(6.027)	(3894.7)			0.0172	
0.1	0.15	0.0937	0.6898	6.817	3556.5	0.831	0.0019	0.0044	−0.0233
				(7.757)	(4193.7)			0.0042	
0.1	0.20	0.1214	0.7162	7.744	4038.8	0.835	0.0019	0.0016	−0.0100
				(8.751)	(4373.0)			0.0008	
1	0	0	0	1.987	11.177	0.992	0.1509	0.0001	−0.0065
				(1.996)	(35.05)			0.0090	
1	0.10	0.0504	0.6684	6.193	34.39	0.982	0.1526	0.0001	−0.0046
				(6.171)	(55.32)			0.0017	
1	0.20	0.0979	0.7736	9.258	55.04	0.980	0.1535	*	−0.0011
				(9.177)	(60.88)			0.0001	
10	0	0	0	1.999	0.2265	0.999	0.8982	*	−0.0002
				(1.999)	(0.675)			0.0002	
10	0.10	0.0489	0.6887	6.601	0.6623	0.998	0.9088	*	−0.0001
				(6.503)	(1.110)			*	
10	0.20	0.0954	0.8290	12.156	1.004	0.998	0.9170	*	*
				(11.959)	(1.366)			*	
100	0	0	0	2.000	0.0741	0.9999	0.9648	*	*
				(2.000)	(0.0765)			*	
100	0.20	0.0952	0.9321	30.442	0.2991	0.9999	0.9903	*	*
				(30.244)	(0.1076)			*	
1000	0	0	0	2.000	0.0672	1.000	0.9675	*	*
				(2.000)	(0.0672)			*	

For the Markov model (Eq. 1) of Colquhoun and Hawkes (1982) with parameter values (Eq. 32) a selection of properties of the observed gateway process $\{(\tilde{J}_k, \tilde{T}_k)\}$ is presented. These are the probabilities $\tilde{p}_o^U$ ($\tilde{p}_c^U$) of a visit to the open (closed) class being undetected, the mean $\tilde{\mu}_o^{(1)}$ ($\tilde{\mu}_c^{(1)}$) and corresponding standard deviation $\tilde{\sigma}_o$ ($\tilde{\sigma}_c$) of an observed open-time (closed-time) (see Eq. 31 and following remarks), the probability $\tilde{\pi}_2$ that an observed opening begins in state 2, the proportion $\tilde{\pi}_o(a) = \tilde{\mu}_o^{(1)}/(\tilde{\mu}_o^{(1)} + \tilde{\mu}_c^{(1)})$ of time the process is observed to be in the open states, and the first two auto-correlations $\tilde{\rho}_{oo}^{(1)}$ and $\tilde{\rho}_{cc}^{(1)}$ and cross-correlation $\tilde{\rho}_{oc}^{(0)}$. *Value whose first four decimals are zero.

to the concentration with the consequence that transitions from 1 to 4 become less frequent; for low concentrations a sojourn in $\mathcal{O}$ beginning in 1 (and exiting to 4) has a non-negligible chance of non-detection but this becomes almost irrelevant for large a. As a increases more and more of the open-closed transitions occur through states 2 and 3. Sojourns in state 3 are generally short, with a consequent substantial probability of non-detection, while those in 2 are 'longer' with a small probability of non-detection. The probability of a class sojourn being undetected depends very little on a for the open class, but is very dependent on a for the closed class. As expected the equilibrium probability of being in the open-class $\mathcal{O} = \{1, 2\}$ increases with concentration, particularly in the interval 10–100 μM. However, this probability is only weakly dependent on τ. This is an important result as it implies that the use of such single channel dose-response curves to estimate the equilibrium properties of channel gating models is robust or relatively insensitive to errors arising from ignoring time interval omission. As could be anticipated, sojourn time auto- and cross-correlations decrease with increasing concentration a and also, though to a lesser extent, with increasing τ.

Without time interval omission a sojourn time in the open (closed) states has a distribution which is a linear combination of two (three) exponential distributions. While this does not hold with time interval omission, as can be inferred from Eq. 19, there are many examples where it remains approximately true (after a time shift τ) and in some cases, such as when $\tilde{\sigma} \approx \tilde{\mu} - \tau$, even a single exponential fits satisfactorily (Milne et al., 1988; Yeo et al., 1988). As concentration increases in the present example, open-time sojourns but not so much closed-time sojourns appear more exponential.

For models with a single gateway, such as the alternating renewal models of Milne et al. (1988), the mean open-time $\mu_o^{(1)}$ is approximately inversely proportional

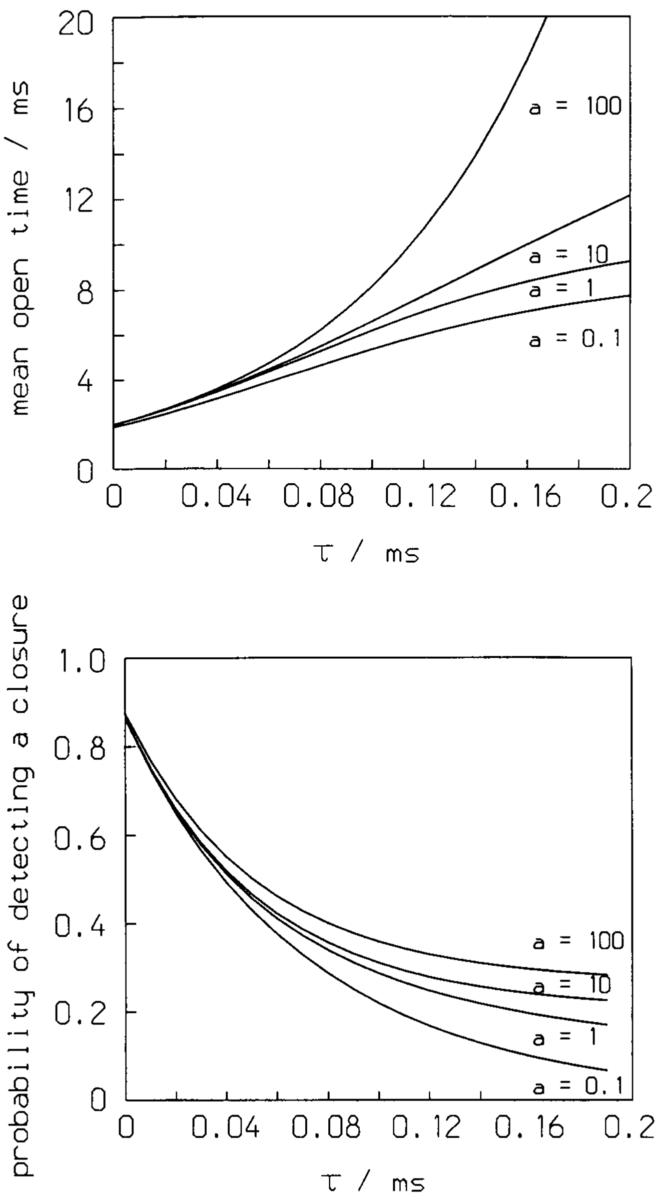

FIGURE 3 Mean observed open-times under time interval omission. For the Markov model (Eq. 1) of Colquhoun and Hawkes (1982) with parameter values given by Eq. 32, the plots show (a) the mean observed open-time $\tilde{\mu}_o^{(1)}$ (see p. 366) and (b) the probability p_c^D of detection of a visit to the closed states $\mathcal{C}$, as a function of the detection limit τ (ms) for various values of the agonist concentration a (μM).

to the probability $p_c^D(\tau)$ that a closed-time is detected. For a single closed state, $p_c^D(\tau)$ usually decreases monotonically towards zero, while for several closed states there is more likely to be an effective lower limit for $p_c^D(\tau)$ (Milne et al., 1988, Figs. 4 and 2); these give different curves and both types can be seen in Fig. 3 particularly for low concentrations.

Although several authors have alluded to the presence of non-Markov structure in single channel experiments, there is a paucity of such models with postulated or estimated parameter values. As an indication of how our semi-Markov program can be used, we have taken model 1 with parameter values as given in Eq. 32, but with states 5 and 4 condensed into a single state 4 having a sojourn time distribution as the appropriate linear combination of two exponentials. The system is then described by a four-state semi-Markov model. The semi-Markov program gives the same numerical results as the full five-state Markov model, as they have identical gateway processes; however, the underlying four-state semi-Markov model is not the same as the gateway process. Any or all of the 4 or 5 states could be given any well-defined sojourn time distributions, for example, Weibull distributions as occur in "fractal" models.

Nagy (1987) has given some partial non-Markov models. If the 'sink' $\mathcal{J}$ of Nagy (1987, p. 257) is permitted eventually to transit to the set $\mathcal{R}$ of resting states, then the system may be approximated by a semi-Markov model which is effectively an alternating renewal model, with an open class $\mathcal{O}$ having sojourn-time density function given by Eq. 1 of that paper, and a closed class $\mathcal{C}$ made up of the inactivated, resting and closed states. Then, if estimates were obtained for the appropriate parameter values, model properties could be readily calculated using our program.

DISCUSSION

We have provided a semi-Markov framework for analysing the dynamic properties of single ion channel behavior, both with and without time interval omission. Two important features of our framework are (a) its generality and (b) its invariance to time interval omission. First, semi-Markov models include most commonly used ion channel models as special cases, in particular Markov, fractal and diffusion models. It is important to note that semi-Markov models permit non-exponential state sojourn time distributions and correlations between successive class (i.e., open or closed) sojourns. Failure to admit the latter is an obvious shortcoming of fractal models. Diffusion models are actually Markov models with a large state space. However, the number of gateway states in such models is usually small, so the corresponding gateway processes have a small state space. This can lead to considerable conceptual and computational simplifications when studying such diffusion models. Secondly, the invariance of our semi-Markov gateway process to time interval omission leads to efficient calculation of channel properties, both with and without time interval omission. This invariance also clarifies the effect of time interval omission, in that it is reduced to a transformation from one semi-Markov kernel to another. For example, it enables us to determine whether inferences concerning the structure of the underlying channel gating process are likely to be robust to time interval omission. Inferences whose origin is in the structure of the gateway process semi-Markov kernel (e.g., those based on auto- and cross-correlation functions) are likely to be robust to time interval omission (Ball and Sansom, 1988b), while inferences whose origin in in the form of the semi-Markov kernel (e.g., those based on open and closed sojourn time pdfs) are less so (Ball, 1990).

When modeling time interval omission, we have assumed that the detection limits for open and closed sojourns are constant and equal. It is straightforward to modify our framework to incorporate non-equal detection limits, and to permit the detection limits to be random variables. We have also assumed, for mathematical convenience, that a detected open (closed) sojourn commences as soon as the channel enters an open (closed) state, rather than after the detection limit τ has elapsed. The latter was adopted in the approaches of Ball and Sansom (1988*a*), Milne et al. (1988), Hawkes et al. (1990), and in Jalali and Hawkes (1992*a*, *b*). In most, if not all, applications it does not really matter which approach is used, since there is also often a simple relationship between model observed channel properties under the two assumptions (Ball et al., 1991).

The formulae in this paper are sufficiently complex to necessitate numerical computation if model channel properties are to be calculated. Such computations can be done in an efficient and structured fashion, as we have indicated. At present we are able to determine equilibrium distributions and sojourn time moments, auto- and cross-correlations, both with and without time interval omission. The main gap in our numerical methodology, as presented here, is the calculation of the semi-Markov kernel of the gateway process incorporating time interval omission. Knowledge of this kernel is important since it would enable us to calculate the pdfs of observed open and closed sojourns. It is an essential part of any likelihood based parameter estimation scheme (see below). The use of numerical solutions of integral equations for calculating the semi-Markov kernel appears to be very promising and will be discussed elsewhere. Approximations to the kernel involving the use of 'phantom states' (Blatz and Magleby, 1986; Crouzy and Sigworth, 1990) may also be fruitful; indeed, in our semi-Markov framework, the sojourn times in such phantom states are no longer constrained to be exponential. If we restrict attention to Markov models then Hawkes et al. (1990) derive a closed form expression for the kernel, which permits accurate computation for small t, though there may be numerical stability problems for large t. However, Jalali and Hawkes (1992*a*, *b*) have developed good approximations in such circumstances. It is important to be able to calculate as many numerical characteristics of single channel behavior as possible, since they greatly aid model validation.

This paper, like many on single channel theory, has been concerned with deriving observable properties of proposed models. However, a topic of considerable importance is the estimation of the parameters of such a model from observed single channel data. Again our semi-Markov framework has an important role to play. The data will consist typically of a sequence $t_1, t_2, \ldots, t_n$ of observed sojourn times, where, for example, t_1 corresponds to an open sojourn, t_2 to a closed sojourn, and so on. Suppose also that the underlying model depends on a vector θ of unknown parameters. Then the likelihood without time interval omission is given, in obvious notation, by

$$L(\theta) = [\pi^{\circ}(\theta)', 0']\left\{\prod_{i=1}^{n} \mathbf{f}(t_i, \theta)\right\}\mathbf{1};$$

see Eq. 4.3 of Fredkin et al. (1985) for the Markov case. Thus θ can, at least in principle, be estimated by the method of maximum likelihood. For Markov models in the absence of time interval omission this has been addressed by Horn and Lange (1983) and Ball and Sansom (1989). When there is time interval omission we again have the problem of calculating efficiently a semi-Markov kernel, now $\tilde{\mathbf{f}}(t, \theta)$, in order to calculate $\tilde{L}(\theta)$. However, it is important to note that several different types of identifiability problem can arise, see for example Fredkin et al. (1985), Yeo et al. (1988), Milne et al. (1989), Kienker (1989), Yang and Swenberg (1992), and Ball et al. (1990). Alternatives to likelihood based inference are attractive, not least because of the difficulty in computing the likelihood. One possible approach is Laplace transform based inference, see, e.g., Feigin et al. (1983) and Laurence and Morgan (1987). Magleby and Weiss (1990*a*, *b*) describe another method, in which the unknown parameters are estimated by matching the model two-dimensional open-closed pdf to that estimated from the data. This method, which is based on an exponential representation of the filter response rather than a discrete detection limit and allows for noise, involves approximating the model open-closed pdf from a large number of simulations, and is highly computationally intensive. If the model open-closed pdf could be expressed in terms of the semi-Markov kernel, $\tilde{\mathbf{f}}(t, \theta)$, then efficient ways of calculating $\tilde{\mathbf{f}}(t, \theta)$ might produce considerable savings in computer time in such applications.

We have developed our framework within the context of equilibrium studies of a single ion channel with two conductance levels. However, many experiments involve alternative conditions, such as more than two conductance levels (e.g., Fredkin and Rice, 1986), multiple channels (e.g., Jackson, 1985; Kijima and Kijima, 1987; Yeo et al., 1989; Dabrowski et al., 1990; and Colquhoun and Hawkes, 1990) or non-equilibrium experiments carried out in the presence of inactivating (or desensitising) channels (e.g., Ball et al., 1989). We are currently investigating the extension of our semi-Markov methodology to these situations. Here we just note that such an extension is relatively straightforward for multiconductance level models and non-equilibrium models containing absorbing states, though it is not clear precisely how time interval omission should be modelled in the former. At a formal level, the simplest models incorporating multiconductance (or subconductance) levels and multiple channel models are mathematically equivalent. However, the experimental questions of interest are likely to be quite different. Again it is far from clear how time interval omission should be modelled in these situations.

APPENDIX

A method for calculating $\mathbf{M}_{\mathcal{U}}^{(r)}$

In this appendix we outline a method, based on integral equations, for numerically calculating $\mathbf{M}_{\mathcal{U}}^{(r)}$ ($r = 0, 1, 2$). Our approach is to first estimate $\mathbf{f}(t)$ ($0 \le t \le \tau$) and then use numerical integration to estimate $\mathbf{M}_{\mathcal{U}}^{(r)}$ via Eq. 25. Recall that $\mathbf{f}(t)$ has Laplace transform $\Phi(\theta)$ given by Eq. 19. Thus

$$\mathbf{f}(t) = \mathbf{L}\mathbf{h}(t)\mathbf{L}^{\mathrm{T}} \quad (t \ge 0),$$

where $\mathbf{h}(t)$ ($t \ge 0$) is the $n \times n$ matrix function with Laplace transform, $\Gamma(\theta)$ say, given by

$$\Gamma(\theta) = (\mathbf{I} - \Psi_0(\theta))^{-1}\Psi_1(\theta) \quad (\theta \ge 0). \tag{A1}$$

Multiplying both sides of Eq. A1 by $\mathbf{I} - \Psi_0(\theta)$ and rearranging, we obtain

$$\Gamma(\theta) = \Psi_1(\theta) + \Psi_0(\theta)\Gamma(\theta) \quad (\theta \ge 0). \tag{A2}$$

Partition $\mathbf{g}(t)$ into

$$\mathbf{g}(t) = \begin{bmatrix} \mathbf{g}_{oo}(t) & \mathbf{g}_{oc}(t) \\ \mathbf{g}_{co}(t) & \mathbf{g}_{cc}(t) \end{bmatrix}$$

and let

$$\mathbf{g}_0(t) = \begin{bmatrix} \mathbf{g}_{oo}(t) & 0 \\ 0 & \mathbf{g}_{cc}(t) \end{bmatrix} \quad \text{and} \quad \mathbf{g}_1(t) = \begin{bmatrix} 0 & \mathbf{g}_{oc}(t) \\ \mathbf{g}_{co}(t) & 0 \end{bmatrix}.$$

Then Eq. A2 can be inverted to yield

$$\mathbf{h}(t) = \mathbf{g}_1(t) + \int_0^t \mathbf{g}_0(u)\mathbf{h}(t-u)\,\mathrm{d}u \quad (t \ge 0). \tag{A3}$$

The integral equation (A3) is multi-dimensional and currently available NAG subroutines cater only for one-dimensional integral equations. However, Eq. A3 can be solved numerically as follows. Define $n \times n$ matrix functions $\mathbf{h}_k(t)$ ($t \ge 0$; $k = 0, 1, \cdots$) by

$$\mathbf{h}_0(t) = \mathbf{g}_1(t),$$

$$\mathbf{h}_k(t) = \mathbf{g}_1(t) + \int_0^t \mathbf{g}_0(u)\mathbf{h}_{k-1}(t-u)\,\mathrm{d}u \quad (k = 1, 2, \cdots).$$

Then it can be shown that, as k tends to infinity, $\mathbf{h}_k(t)$ converges to $\mathbf{h}(t)$ geometrically fast, so $\mathbf{h}(t)$ can be estimated iteratively using numerical integration.

We are grateful to the referees for their helpful comments.

This work was made possible by grants from the Australian Research Council, the Danish Natural Science Research Council and the UK Science and Engineering Research Council.

Received for publication May 1991 and in final form 23 September 1992.

REFERENCES

Ball, F. G. 1990. Aggregated Markov processes with negative exponential time interval omission. *Adv. Appl. Prob.* 22:802–830.

Ball, F. G., S. S. Davies, and M. S. P. Sansom. 1990. Single-channel data and missed events: analysis of two-state Markov model. *Proc. R. Soc. Lond. B Biol. Sci.* 242:61–67.

Ball, F. G., C. J. Kerry, R. L. Ramsey, M. S. P. Sansom, and P. N. R. Usherwood. 1988. The use of dwell time cross-correlation functions to study single-ion channel gating kinetics. *Biophys. J.* 54:309–320.

Ball, F. G., R. McGee, and M. S. P. Sansom. 1989. Analysis of post-perturbation gating kinetics of single ion channels. *Proc. R. Soc. Lond. B Biol. Sci.* 236:29–52.

Ball, F. G., R. K. Milne, and G. F. Yeo. 1991. Aggregated semi-Markov processes incorporating time interval omission. *Adv. Appl. Prob.* 23:772–797.

Ball, F. G., R. K. Milne, and G. F. Yeo. 1993. On the exact distribution of observed open times in single ion channel models. *J. Appl. Prob.* In press.

Ball, F. G., and J. A. Rice. 1989. A note on single-channel autocorrelation functions. *Math. Biosciences.* 97:17–26.

Ball, F. G., and M. S. P. Sansom. 1987. Temporal clustering of ion channel openings incorporating time interval omission. *IMA J. Math. Appl. Med. Biol.* 4:333–361.

Ball, F. G., and M. S. P. Sansom. 1988*a*. Aggregated Markov processes incorporating time interval omission. *Adv. Appl. Prob.* 20:546–572.

Ball, F. G., and M. S. P. Sansom. 1988*b*. Single-channel autocorrelation functions: the effects of time interval omission. *Biophys. J.* 53:819–832.

Ball, F. G., and M. S. P. Sansom. 1989. Ion-channel gating mechanisms: model identification and parameter estimation from single channel recordings. *Proc. R. Soc. Lond. B Biol. Sci.* 236:385–416.

Bates, S. E., M. S. P. Sansom, F. G. Ball, R. L. Ramsey, and P. N. R. Usherwood. 1990. Glutamate receptor-channel gating: maximum likelihood analysis of gigaohm seal recordings from locust muscle. *Biophys. J.* 58:219–229.

Bellman, R. 1960. Introduction to Matrix Analysis. McGraw-Hill, New York.

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

Blatz, A. L., and K. L. Magleby. 1989. Adjacent interval analysis distinguishes among gating mechanisms for the fast chloride channel from rat skeletal muscle. *J. Physiol.* 410:561–585.

Brooks, C. L., M. Karplus, and B. M. Pettitt. 1988. Proteins: A Theoretical Perspective of Dynamics, Structure and Thermodynamics. John Wiley and Sons, New York.

Çinlar, E. 1969. Markov renewal theory. *Adv. Appl. Prob.* 1:123–187.

Çinlar, E. 1975. Introduction to Stochastic Processes. Prentice-Hall, Englewood Cliffs, NJ.

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

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

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

Colquhoun, D., and A. G. Hawkes. 1990. Stochastic properties of ion channel openings and bursts in a membrane patch that contains two channels: evidence concerning the number of channels present when a record containing only single openings is observed. *Proc. R. Soc. Lond. B Biol. Sci.* 240:453–477.

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

Condat, C. A. 1989. Defect-diffusion and closed-time distribution for ionic channels in cell membranes. *Phys. Rev. A.* 39:2112–2125.

Condat, C. A., and J. Jäckle. 1989. Closed-time distribution of ionic channels. Analytical solution to a one-dimensional defect-diffusion model. *Biophys. J.* 55:915–925.

Cox, D. R., and H. D. Miller. 1965. The Theory of Stochastic Processes. Methuen, London.

Crouzy, S. C., and F. J. Sigworth. 1990. Yet another approach to the dwell-time omission problem of single-channel analysis. *Biophys. J.* 58:731–743.

Croxton, T. L. 1988. A model of the gating of ion channels. *Biochim. Biophys. Acta.* 946:19–24.

Dabrowski, A. R., D. McDonald, and U. Rösler. 1990. Renewal properties of ion channels. *Ann. Statist.* 18:1091–1115.

Doster, W., W. Schirmacher, and W. Settles. 1990. Percolation model of ionic channel dynamics. *Biophys. J.* 57:681–684.

Edeson, R. O., G. F. Yeo, R. K. Milne, and B. W. Madsen. 1990. Graphs, random sums, and sojourn time distributions, with application to ion-channel modeling. *Math. Biosciences.* 102:75–104.

Feigin, P. D., R. L. Tweedie, and C. Belyea. 1983. Weighted area techniques for explicit parameter estimation. *Aust. J. Statist.* 25:1–16.

Frauenfelder, H., F. Parak, and R. D. Young. 1988. Conformational substates in proteins. *Annu. Rev. Biophys. Biophys. Chem.* 17:451–479.

Fredkin, D. R., M. Montal, and J. A. Rice. 1985. Identification of aggregated Markovian models: application to the nicotinic acetylcholine receptor. *In* Proceedings of the Berkeley Conference in Honor of Jerzy Neyman and Jack Keifer. Vol. 1. L. M. Le Cam and R. A. Olshen, editors. Wadsworth Publishing Co., Belmont CA. 269–289.

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

Hawkes, A. G., A. Jalali, and D. Colquhoun. 1990. The distributions of the apparent open times and shut times in a single channel record when brief events cannot be detected. *Phil. Trans. R. Soc. Lond. A.* 332:511–538.

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

Jackson, M. B. 1985. Stochastic behavior of a many-channel membrane system. *Biophys. J.* 47:129–137.

Jalali, A., and A. G. Hawkes. 1992*a*. The distribution of apparent occupancy times in a two-state Markov process in which brief events cannot be detected. *Adv. Appl. Prob.* 24:288–301.

Jalali, A., and A. G. Hawkes. 1992*b*. Generalised eigenproblems arising in aggregated Markov processes allowing for time interval omission. *Adv. Appl. Prob.* 24:302–321.

Kerry, C. J., K. S. Kits, R. L. Ramsey, M. S. P. Sansom, and P. N. R. Usherwood. 1987. Single channel kinetics of a glutamate receptor. *Biophys. J.* 51:137–144.

Kerry, C. J., R. L. Ramsey, M. S. P. Sansom, and P. N. R. Usherwood. 1988. Glutamate receptor-channel kinetics: the effect of glutamate concentration. *Biophys. J.* 53:39–52.

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

Kijima, S., and H. Kijima. 1987. Statistical analysis of channel current from a membrane patch II. A stochastic theory of a multi-channel system in the steady-state. *J. Theor. Biol.* 128:435–455.

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

Läuger, P. 1983. Conformational transitions of ionic channels. *In* Single-Channel Recording. B. Sakmann and E. Neher, editors. Plenum Press, New York. 177–189.

Läuger, P. 1988. Internal motions of proteins and gating kinetics of ionic channels. *Biophys. J.* 53:877–884.

Laurence, A. F., and B. J. T. Morgan. 1987. Selection of the transformation variable in the Laplace transform method of estimation. *Aust. J. Statist.* 29:113–127.

Levitan, I. B. 1988. Modulation of ion channels in neurons and other cells. *Annu. Rev. Neurosci.* 11:119–136.

Levitt, D. G. 1989. Continuum model of voltage-dependent gating: macroscopic conductance, gating current, and single-channel behaviour. *Biophys. J.* 55:489–498.

Liebovitch, L. S., J. Fischbarg, and J. P. Koniarek. 1987. Ion channel kinetics: a model based on fractal scaling rather than multistate Markov processes. *Math. Biosciences.* 84:37–68.

Liebovitch, L. S., and J. M. Sullivan. 1987. Fractal analysis of a voltage-dependent potassium channel from cultured mouse hippocampal neurons. *Biophys. J.* 52:979–988.

McManus, O. B., A. L. Blatz, and K. L. Magleby. 1985. Inverse relationship of the durations of adjacent open and shut intervals for Cl and K channels. *Nature(Lond.).* 317:625–627.

McManus, O. B., and K. L. Magleby. 1989. Kinetic time constants independent of previous single channel activity suggest Markov gating for a large conductance Ca-activated K channel. *J. Gen. Physiol.* 57:1037–1070.

Magleby, K. L., and D. S. Weiss. 1990*a*. Identifying kinetic gating mechanisms for ion channels by using two-dimensional distributions of simulated dwell times. *Proc. Roy. Soc. Lond. B Biol. Sci.* 241:220–228.

Magleby, K. L., and D. S. Weiss. 1990*b*. Estimating kinetic parameters for single channels with simulation. A general method that resolves the missed event problem and accounts for noise. *Biophys. J.* 58:1411–1426.

Millhauser, G. L. 1990. Reptation theory of ion channel gating. *Biophys. J.* 57:857–864.

Millhauser, G. L., E. E. Salpeter, and R. E. Oswald. 1988*a*. Diffusion models of ion-channel gating and the origin of power-law distributions from single-channel recording. *Proc. Natl. Acad. Sci. USA.* 85:1503–1507.

Millhauser, G. L., E. E. Salpeter, and R. E. Oswald. 1988*b*. Rate-amplitude correlation from single-channel records: a hidden structure in ion channel gating kinetics? *Biophys. J.* 54:1165–1168.

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

Milne, R. K., G. F. Yeo, B. W. Madsen, and R. O. Edeson. 1989. Estimation of single channel kinetic parameters from data subject to limited time resolution. *Biophys. J.* 55:673–676.

Moczydlowski, E. 1986. Single-channel enzymology. *In* Ion Channel Reconstitution. C. Miller, editor. Plenum Press, New York. 75–113.

Moler, C., and C. Van Loan. 1978. Nineteen dubious ways to compute the exponential of a matrix. *SIAM (Soc. Ind. Appl. Math.) Rev.* 20:801–836.

Nagy, K. 1987. Evidence for multiple open states of sodium channels in neuroblastoma cells. *J. Memb. Biol.* 96:251–262.

Oswald, R. E., G. L. Millhauser, and A. A. Carter. 1991. Diffusion model in ion channel gating: extension to agonist-activated ion channels. *Biophys. J.* 59:1136–1142.

Pyke, R. 1961. Markov renewal processes: definitions and preliminary properties. *Ann. Math. Statist.* 32:1231–1242.

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

Sansom, M. S. P., F. G. Ball, C. J. Kerry, R. M. Ramsey, R. L. Ramsey, and P. N. R. Usherwood. 1989. Markov, fractal, diffusion and related models of ion channel gating: a comparison with experimental data from two ion channels. *Biophys. J.* 56:1229–1243.

Yang, G. L., and C. E. Swenberg. 1992. Estimation of open dwell time and problems of identifiability in channel experiments. *J. Statist. Plan. Inf.* In press.

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

Yeo, G. F., R. O. Edeson, R. K. Milne, and B. W. Madsen. 1989. Superposition properties of independent ion channels. *Proc. Roy. Soc. Lond. B Biol. Sci.* 238:155–170.