Joint distributions of apparent open and shut times of single-ion channels and maximum likelihood fitting of mechanisms

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Contents

The openings and shuttings of individual ion channel molecules can be modelled in terms of an underlying Markov process with discrete states in continuous time. In practice, some of the open times, and/or shut times, are too short to be detected reliably, making the durations of some of these intervals appear to be longer than they really are. Under certain assumptions about how this happens, the probability densities of these apparent times have previously been obtained. It has been shown that the ability to distinguish between alternative postulated reaction mechanisms can be greatly improved by considering bivariate distributions. In this paper we obtain joint distributions, and hence conditional distributions, of adjacent apparent open and shut times. Numerical examples illustrate what insight these conditional distributions may provide about the underlying mechanism. Bivariate distributions are readily generalized to multivariate distributions which enable the likelihood for an entire single-channel recording to be computed, and hence efficient maximum likelihood estimates for the mechanism's rate constants can be obtained. Numerical examples of such fitting are given.

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1. Introduction

Single channel records always seem to show phenomena that are just too rapid too be resolved easily, whatever efforts are made to increase the resolution. With present techniques, an opening of the ion channel that is shorter than about 25 μs will not be detectable, even in the best records, given the noise in the recording. The resolution is usually considerably worse than this, to an extent that depends on the signal-to-noise ratio of the recording and on the method used for its analysis (see Colquhoun & Sigworth (1995) for details). Events (openings or shuttings) of the channel that have a duration much shorter than the resolution will not be detected, and the results will therefore be distorted to an extent that can be quite serious in cases of practical interest.

In this paper we address the problem of obtaining joint and conditional distributions of the adjacent *apparent* open and shut times that are recorded when brief events are missed, and their use for direct maximum likelihood fitting of entire data records. This is an important problem, because Fredkin *et al.* (1985) showed that, when the data show correlations, it is not possible to extract all the information from the experimental record by examination of only the univariate distributions (e.g. of open times, or of shut times), but it is necessary also to exploit bivariate distributions (e.g. that of open time and adjacent shut time). It was subsequently demonstrated very clearly by Magleby and co-workers that the ability to distinguish between alternative postulated reaction mechanisms can be greatly improved by exploitation of the correlational information contained in bivariate distributions (McManus *et al.* 1985; Blatz & Magleby 1989; Magleby & Weiss 1990*b*; Magleby & Song 1992). Although some of the relevant information can be recovered by measuring correlation coefficients (e.g. between the length of one opening and the length of the next opening) (Fredkin *et al.* 1985; Colquhoun & Sakmann 1985; Colquhoun & Hawkes 1987), the graphical displays are generally preferable. For example, one can plot the mean open time conditional on the length of the adjacent shut time (see, for example, Blatz & Magleby 1989; Gibb & Colquhoun 1992). These bivariate and conditional distributions can be predicted, on the basis of any postulated mechanism, for comparison with experimental data, but in order for these methods to be useful with real experimental data, they need to be extended to allow for limited ability to detect brief events; that is the purpose of this paper. Once such distributions have been obtained the door is opened to doing direct maximum likelihood fits of a reaction mechanism to experimental data (see Sine *et al.* 1990), and we shall also discuss this problem.

At this point it is worth noting that it is quite possible for observations to exhibit correlations when in fact the underlying channel mechanism predicts that there should be none. The fact that time resolution is limited can itself produce spurious correlations under some circumstances (Srodzinski 1994; Colquhoun & Hawkes 1995*a*, p. 461). This fact provides another good reason for making correct allowance for missed events when analysing real data. Another hazard arises from the fact that it is also possible for spurious correlations to arise if the recording is made from a heterogeneous population of channels.

The definition of an *apparent open time* used here, and in most other work on the subject, is as follows. If a fixed dead-time ξ is assumed then an *apparent opening* is defined as starting with an opening of duration of at least ξ followed by any number of openings and shuttings, all the shut times being shorter than ξ; the apparent opening ends when a shut time longer than ξ occurs. A similar definition is used for apparent shut times. Open times defined in this way will be referred to as *e*-open times (*extended* openings). This definition should give a good approximation to the values that are measured from an experimental record in most cases (though this is, to some extent, dependent on what method is used for measuring the record).

Several approximate methods have been described for coping with this 'missed event' problem (see, for example, Roux & Sauvé 1955; Blatz & Magleby 1986; Yeo *et al.* 1988; Crouzy & Sigworth 1990). Ball & Sansom (1988*a*) obtained the distribution of apparent openings and shuttings in terms of Laplace transforms. The exact probability density was found by Hawkes *et al.* (1990) and very accurate asymptotic forms were obtained by Jalali & Hawkes (1992*a*, *b*) and further discussed in Hawkes *et al.* (1992).

It is supposed in what follows that all events that are shorter than some fixed *resolution* or *dead-time* (denoted ξ) are *not* detected, while all events that are longer than ξ are detected and measured accurately. The resolution is usually not well defined, so it must be imposed retrospectively on the measurements by, for example, concatenating any observed shut time below ξ with the open times on each side of it to produce one long 'apparent opening' (Colquhoun & Sigworth 1995). This will happen automatically with very short shut times which will not be observed anyway. Short openings are similarly treated to obtain 'apparent shut times'.

Although the expressions that are derived here are obviously more complicated than in the ideal ($\xi = 0$) case, their numerical evaluation requires no new techniques (see Colquhoun & Hawkes (1995*b*) for an introduction).

2. Notation and basic results

The principles and notation are those employed by Hawkes *et al.* (1990, 1992). The underlying system is modelled by a finite-state Markov process, $X(t)$, in continuous time; $X(t) = i$ denotes the system is in state i at time t. The rate constants for transitions between states i and j ($i \neq j$) are the elements, q_{ij}, of the transition rate matrix $\boldsymbol{Q}$, and the diagonal elements, q_{ii}, are defined so that the rows sum to zero.

The ideal case

If the k states of the system are divided into subset $\mathcal{A}$ containing the open states, $k_{\mathcal{A}}$ in number, and subset $\mathcal{F}$ containing the shut states, $k_{\mathcal{F}}$ in number, so $k_{\mathcal{A}}+k_{\mathcal{F}} = k$, then the $\boldsymbol{Q}$ matrix may be partitioned as

$$\boldsymbol{Q} = \begin{bmatrix} \boldsymbol{Q}_{\mathcal{AA}} & \boldsymbol{Q}_{\mathcal{AF}} \\ \boldsymbol{Q}_{\mathcal{FA}} & \boldsymbol{Q}_{\mathcal{FF}} \end{bmatrix}. \tag{2.1}$$

A semi-Markov process (for an elementary introduction see, for example, Cox & Miller 1965, ch. 9) is embedded in the process at the instants at which the system enters the set $\mathcal{A}$ or $\mathcal{F}$. The intervals between these points have probability densities given by the matrix

$$\boldsymbol{G}(t) = \begin{bmatrix} \mathbf{0} & \exp(\boldsymbol{Q}_{\mathcal{AA}}t)\boldsymbol{Q}_{\mathcal{AF}} \\ \exp(\boldsymbol{Q}_{\mathcal{FF}}t)\boldsymbol{Q}_{\mathcal{FA}} & \mathbf{0} \end{bmatrix}. \tag{2.2}$$

Thus each event is, alternately, the beginning of an open period or the beginning of a closed period. The elements, $g_{ij}(t)$, of the top right-hand corner of this matrix give the probability density for staying within the open states (set $\mathcal{A}$) for a time t *and* then leaving for shut state j, conditional on starting in open state i (see, for details, Colquhoun & Hawkes 1982). The Laplace transform of this matrix will be denoted by

$$\boldsymbol{G}^*(s) = \begin{bmatrix} \mathbf{0} & \boldsymbol{G}^*_{\mathcal{AF}}(s) \\ \boldsymbol{G}^*_{\mathcal{FA}}(s) & \mathbf{0} \end{bmatrix}, \tag{2.3}$$

where

$$\boldsymbol{G}^*_{\mathcal{AF}}(s) = (s\boldsymbol{I} - \boldsymbol{Q}_{\mathcal{AA}})^{-1}\boldsymbol{Q}_{\mathcal{AF}}, \quad \boldsymbol{G}^*_{\mathcal{FA}}(s) = (s\boldsymbol{I} - \boldsymbol{Q}_{\mathcal{FF}})^{-1}\boldsymbol{Q}_{\mathcal{FA}}. \tag{2.4}$$

The Markov chain embedded at these open–shut transition points, ignoring the duration of the intervals between them, has transition probability matrix

$$\boldsymbol{G} = \begin{bmatrix} \mathbf{0} & \boldsymbol{G}_{\mathcal{AF}} \\ \boldsymbol{G}_{\mathcal{FA}} & \mathbf{0} \end{bmatrix}, \tag{2.5}$$

where

$$\boldsymbol{G}_{\mathcal{AF}} = \int_0^\infty \boldsymbol{G}_{\mathcal{AF}}(t)\,\mathrm{d}t = \boldsymbol{G}^*_{\mathcal{AF}}(0) = -\boldsymbol{Q}^{-1}_{\mathcal{AA}}\boldsymbol{Q}_{\mathcal{AF}},$$

$$\boldsymbol{G}_{\mathcal{FA}} = \int_0^\infty \boldsymbol{G}_{\mathcal{FA}}(t)\,\mathrm{d}t = \boldsymbol{G}^*_{\mathcal{FA}}(0) = -\boldsymbol{Q}^{-1}_{\mathcal{FF}}\boldsymbol{Q}_{\mathcal{FA}}. \tag{2.6}$$

Thus, for example, $\boldsymbol{G}_{\mathcal{AF}}$ has elements that give the probability of leaving the set of open states for shut state j, conditional on starting in open state i, regardless of how long it takes for this transition to occur.

Colquhoun & Hawkes (1982), using the above results as a starting point, went on to study the dynamics of opening and shutting in some detail.

The case of limited time resolution

To modify the above ideas so as to describe the apparent open and shut times, allowing for the omission of short intervals as described in the previous section, we follow Ball & Sansom (1988*a*) by considering a semi-Markov process, the events of which occur at time ξ after the start of observed open or closed periods. An *event type* (open or shut) will be the state of the underlying Markov process, $X(t)$, which is occupied at that time. The durations of the intervals between events, which we call e-open and e-closed intervals, are, according to the previous definition, the same as the durations of the *observed*, or *apparent*, open and closed intervals, because we have taken the same ξ to detect both open and closed periods. The only difference therefore is that the whole process is shifted back by a constant ξ. This makes the theory somewhat easier, because at the event times so defined we know whether there is an apparent open time or apparent shut time in progress. The theory can be done directly in terms of the apparent intervals, but it is not quite so elegant. These definitions are illustrated in figure 1.

Intervals of this process will be alternately e-open and e-closed, so the transition densities, analogous with those in (2.3), will be given by a matrix of the form

$${}^e\boldsymbol{G}(t) = \begin{bmatrix} \mathbf{0} & {}^e\boldsymbol{G}_{\mathcal{AF}}(t) \\ {}^e\boldsymbol{G}_{\mathcal{FA}}(t) & \mathbf{0} \end{bmatrix}, \tag{2.7}$$

with Laplace transform

$${}^e\boldsymbol{G}^*(s) = \begin{bmatrix} \mathbf{0} & {}^e\boldsymbol{G}^*_{\mathcal{AF}}(s) \\ {}^e\boldsymbol{G}^*_{\mathcal{FA}}(s) & \mathbf{0} \end{bmatrix}. \tag{2.8}$$

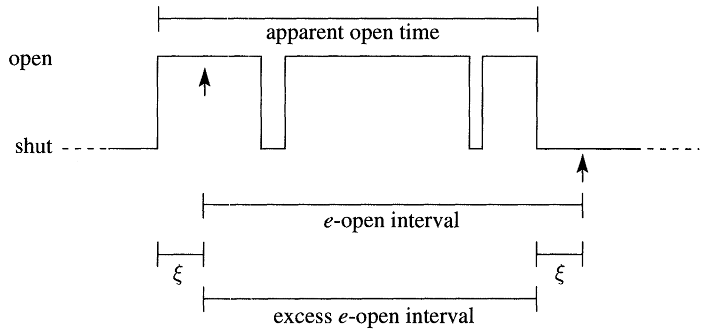


Figure 1. Illustration of the definition of apparent open time which contains two short shut times (of duration less than ξ). The e-open time is defined to start at time ξ after the start of the apparent time, and end time ξ after it finishes, and is thus the same length.

The Markov chain embedded at the event points (the times at which events occur) has transition matrix

$$ {}^e\boldsymbol{G} = \begin{bmatrix} \mathbf{0} & {}^e\boldsymbol{G}_{\mathcal{AF}} \\ {}^e\boldsymbol{G}_{\mathcal{FA}} & \mathbf{0} \end{bmatrix}. \tag{2.9} $$

As in equations (2.5), (2.6), we simplify the notation when setting $s = 0$ in a Laplace transform by omitting the '$*$' and the argument: for example, in this case ${}^e\boldsymbol{G}^*_{\mathcal{AF}}(0)$ is written as ${}^e\boldsymbol{G}_{\mathcal{AF}}$. As above, this gives probabilities for transitions from apparently open to apparently shut states regardless of when the transition occurs.

The equilibrium vector

The distribution of e-open times that will be obtained later requires that we specify the probability that an e-opening starts in each of the open (set $\mathcal{A}$) states. The vector, $\phi_{\mathcal{A}}$, containing these probabilities can be found, for a record at equilibrium, as follows. By looking only at alternate events, and ignoring the interval durations, we have a Markov chain on the $\mathcal{A}$ states with transition matrix ${}^e\boldsymbol{G}_{\mathcal{AF}}\,{}^e\boldsymbol{G}_{\mathcal{FA}}$, and an equilibrium probability vector, $\phi_{\mathcal{A}}$, that satisfies

$$ \phi_{\mathcal{A}} = \phi_{\mathcal{A}}\,{}^e\boldsymbol{G}_{\mathcal{AF}}\,{}^e\boldsymbol{G}_{\mathcal{FA}}, \quad \phi_{\mathcal{A}}\boldsymbol{u}_{\mathcal{A}} = 1, \tag{2.10} $$

where $\boldsymbol{u}_{\mathcal{A}}$ is a vector, the elements of which, $k_{\mathcal{A}}$ in number, are all unity. Once we have a method for evaluating ${}^e\boldsymbol{G}_{\mathcal{AF}}$ and ${}^e\boldsymbol{G}_{\mathcal{FA}}$, this equation can be solved for $\phi_{\mathcal{A}}$ using one of the methods described by Colquhoun & Hawkes (1995*b*) for obtaining the equilibrium state occupancies from the $\boldsymbol{Q}$ matrix (the equation for which is similar to (2.10)).

Similarly, a Markov chain at the closed events has transition matrix ${}^e\boldsymbol{G}_{\mathcal{FA}}\,{}^e\boldsymbol{G}_{\mathcal{AF}}$ with equilibrium vector

$$ \phi_{\mathcal{F}} = \phi_{\mathcal{A}}\,{}^e\boldsymbol{G}_{\mathcal{AF}}. \tag{2.11} $$

Distribution of e-open lifetimes

We will discuss the probability density function of e-open times; the distribution of e-closed times can be obtained simply by interchanging $\mathcal{A}$ and $\mathcal{F}$ in the results. Let ${}^{\mathcal{A}}\boldsymbol{R}(u)$ be a matrix, the ijth element ($i,\, j \in \mathcal{A}$) of which is

$$ {}^{\mathcal{A}}\boldsymbol{R}_{ij}(u) = P[X(u) = j \text{ and no shut time is detected over } (0,u)|X(0) = i], \tag{2.12} $$

where a detectable shut time is a sojourn in $\mathcal{F}$ of duration greater than ξ. This is a kind of reliability or survivor function: it gives the probability that an e-open time, starting in state i, has not yet finished after time u and is currently in state j. Then the transition density is given by

$$ {}^{e}\boldsymbol{G}_{\mathcal{AF}}(t) = {}^{\mathcal{A}}\boldsymbol{R}(t-\xi)\boldsymbol{Q}_{\mathcal{AF}}\exp(\boldsymbol{Q}_{\mathcal{FF}}\xi), \quad t \geqslant \xi, \tag{2.13} $$

because, for the e-open interval to end at time t, there must be a transition from $\mathcal{A}$ to $\mathcal{F}$ at time $t-\xi$ (with no detectable sojourn in $\mathcal{F}$ up to that time), followed by a sojourn of at least ξ in $\mathcal{F}$. The probability density of observed open times is given by

$$ f_{T_0}(t) = \boldsymbol{\phi}_{\mathcal{A}}\, {}^{e}\boldsymbol{G}_{\mathcal{AF}}(t)\boldsymbol{u}_{\mathcal{F}} = \boldsymbol{\phi}_{\mathcal{A}}\, {}^{\mathcal{A}}\boldsymbol{R}(t-\xi)\boldsymbol{Q}_{\mathcal{AF}}\exp(\boldsymbol{Q}_{\mathcal{FF}}\xi)\boldsymbol{u}_{\mathcal{F}}, \quad t \geqslant \xi, \tag{2.14} $$

where $\boldsymbol{u}_{\mathcal{F}}$ is a vector, the elements of which, $k_{\mathcal{F}}$ in number, are all unity. Examples of distributions of observed open and shut times, with various resolutions, are given later (figures 5 and 6).

The distribution of e-open times covers the range $t = \xi$ to ∞ because, by definition, any e-open time, T_0 say, must exceed ξ in duration. Therefore, for these two densities, and all others subsequently quoted in this paper (except (2.15)), we take it as implicit that the density is zero for $t < \xi$. It may sometimes be more convenient to consider the excess time $U_0 = T_0 - \xi$, which ranges from 0 to ∞. We will call this the excess e-open interval (see figure 1). Then the probability density $f_{T_0}(t) = f_{U_0}(t-\xi)$ and so

$$ f_{U_0}(u) = f_{T_0}(u+\xi) = \boldsymbol{\phi}_{\mathcal{A}}\, {}^{e}\boldsymbol{G}_{\mathcal{AF}}(u+\xi)\boldsymbol{u}_{\mathcal{F}} = \boldsymbol{\phi}_{\mathcal{A}}\, {}^{\mathcal{A}}\boldsymbol{R}(u)\boldsymbol{Q}_{\mathcal{AF}}\exp(\boldsymbol{Q}_{\mathcal{FF}}\xi)\boldsymbol{u}_{\mathcal{F}}. \tag{2.15} $$

The problem of evaluating this distribution of apparent open times, and the other distributions derived here, reduces to the problem of evaluating the survivor function, ${}^{\mathcal{A}}\boldsymbol{R}(u)$, and its analogue for apparent shut times ${}^{\mathcal{F}}\boldsymbol{R}(u)$. This will be described next.

Exact evaluation of the survivor function ${}^{\mathcal{A}}\boldsymbol{R}(u)$

Hawkes *et al.* (1990) show that the Laplace transform of ${}^{\mathcal{A}}\boldsymbol{R}(u)$ can be written as

$$ {}^{\mathcal{A}}\boldsymbol{R}^*(s) = \{\boldsymbol{I} - \boldsymbol{G}^*_{\mathcal{AF}}(s)\boldsymbol{S}^*_{\mathcal{FF}}(s)\boldsymbol{G}^*_{\mathcal{FA}}(s)\}^{-1}(s\boldsymbol{I} - \boldsymbol{Q}_{\mathcal{AA}})^{-1}, \tag{2.16} $$

where $\boldsymbol{S}^*_{\mathcal{FF}}(s)$ is defined by the equation

$$ \begin{aligned} \int_0^{\xi} \mathrm{e}^{-st}\exp(\boldsymbol{Q}_{\mathcal{FF}}t)\,\mathrm{d}t &= \{\boldsymbol{I} - \exp(-(s\boldsymbol{I} - \boldsymbol{Q}_{\mathcal{FF}})\xi)\}(s\boldsymbol{I} - \boldsymbol{Q}_{\mathcal{FF}})^{-1} \\ &= \boldsymbol{S}^*_{\mathcal{FF}}(s)(s\boldsymbol{I} - \boldsymbol{Q}_{\mathcal{FF}})^{-1}. \end{aligned} \tag{2.17} $$

When multiplied by $\boldsymbol{Q}_{\mathcal{FA}}$, this is just the Laplace transform of the shut time densities for shut times that are shorter than ξ, which are the elements of

$$ \exp(\boldsymbol{Q}_{\mathcal{FF}}t)\boldsymbol{Q}_{\mathcal{FA}}(1 - H(t-\xi)), $$

where $H(t)$ is a unit step function. Thus the expression

$$ \boldsymbol{G}^*_{\mathcal{AF}}(s)\boldsymbol{S}^*_{\mathcal{FF}}(s)\boldsymbol{G}^*_{\mathcal{FA}}(s) = \boldsymbol{G}^*_{\mathcal{AF}}(s)\mathcal{L}[\exp(\boldsymbol{Q}_{\mathcal{FF}}t)\boldsymbol{Q}_{\mathcal{FA}}(1 - H(t-\xi))] $$

(where $\mathcal{L}$ denotes a Laplace transform) convolves openings (of any length) with shut times that are less than ξ.

Equation (2.16) was given by Ball & Sansom (1988*a*), using different notation. Substituting (2.17) into (2.16), and using (2.4) yields the alternative expression

$$ {}^{\mathcal{A}}\boldsymbol{R}^*(s) = \left[s\boldsymbol{I} - \boldsymbol{Q}_{\mathcal{AA}} - \boldsymbol{Q}_{\mathcal{AF}}\left(\int_0^{\xi}\mathrm{e}^{-st}\exp(\boldsymbol{Q}_{\mathcal{FF}}t)\,\mathrm{d}t\right)\boldsymbol{Q}_{\mathcal{FA}}\right]^{-1}. \tag{2.18} $$

When the resolution is perfect, $\xi = 0$, then $\boldsymbol{S}^*_{\mathcal{FF}}(s) = 0$, ${}^{\mathcal{A}}\boldsymbol{R}(u) = \exp(Q_{\mathcal{AA}}u)$, and all the results reduce to those given by Colquhoun & Hawkes (1982).

Hawkes *et al.* (1990) inverted the Laplace transform in (2.16), and hence obtained an exact solution for the PDF, $f_{T_0}(t)$. The solution has a different form for each of the intervals $t = 0 \rightarrow \xi$, $t = \xi \rightarrow 2\xi$, etc. The solutions are not, as in the ideal case, mixtures of $k_{\mathcal{A}}$ exponentials, but they are products of polynomials in t (of degree that increases for each successive interval) and exponentials; furthermore, the exponential terms involve not the $k_{\mathcal{A}}$ eigenvalues of $\boldsymbol{Q}_{\mathcal{AA}}$, but the k eigenvalues of $\boldsymbol{Q}$. However, the precision of the asymptotic solution (see below) is such that the exact solution need be calculated only for the first three intervals, and for these the exact solution is relatively simple. We therefore give below the exact results for apparent open times in the ranges $t = 0 \rightarrow \xi$, $t = \xi \rightarrow 2\xi$ and $t = 2\xi \rightarrow 3\xi$, which turn out to be adequate in practice.

The starting point is the representation of the matrix $\boldsymbol{Q}$ in terms of the spectral matrices A_i (see, for example, Colquhoun & Hawkes 1982), so that, assuming the matrix $-\boldsymbol{Q}$ has distinct eigenvalues λ_i,

$$\exp(\boldsymbol{Q}t) = \sum_{i-1}^{k} A_i \exp(-\lambda_i t). \tag{2.19}$$

Let $A_{i\mathcal{AF}}$ be the $\mathcal{AF}$ partition of A_i, and define

$$\boldsymbol{D}_i = A_{i\mathcal{AF}} \exp(\boldsymbol{Q}_{\mathcal{FF}}\xi)\boldsymbol{Q}_{\mathcal{FA}}. \tag{2.20}$$

Then ${}^{\mathcal{A}}\boldsymbol{R}(u)$ is given by

$$\left.\begin{aligned} {}^{\mathcal{A}}\boldsymbol{R}(u) &= \boldsymbol{N}_0(u), && 0 \leqslant u \leqslant \xi, \\ &= \boldsymbol{N}_0(u) - \boldsymbol{N}_1(u-\xi), && \xi \leqslant u \leqslant 2\xi, \end{aligned}\right\} \tag{2.21}$$

where

$$\boldsymbol{N}_0(u) = \sum_{i=1}^{k} \boldsymbol{C}_{i00} \exp(-\lambda_i u), \quad \boldsymbol{N}_1(u) = \sum_{i=1}^{k} (\boldsymbol{C}_{i10} + \boldsymbol{C}_{i11}u) \exp(-\lambda_i u), \tag{2.22}$$

and the matrices $\boldsymbol{C}_{imr}$ are given recursively by

$$\boldsymbol{C}_{i00} = A_{i\mathcal{AA}}, \quad \boldsymbol{C}_{i10} = \sum_{j\neq i} (\boldsymbol{D}_i\boldsymbol{C}_{j00} + \boldsymbol{D}_j\boldsymbol{C}_{i00})/(\lambda_j - \lambda_i), \quad \boldsymbol{C}_{i11} = \boldsymbol{D}_i\boldsymbol{C}_{i00}. \tag{2.23}$$

Thus the PDF of the apparent open times, defined in (2.14), can be written as

$$\begin{aligned} f_{T_0}(t) &= 0, \quad 0 \leqslant t \leqslant \xi \\ &= f_0(t-\xi), \quad \xi \leqslant t \leqslant 2\xi \\ &= f_0(t-\xi) - f_1(t-2\xi), \quad 2\xi \leqslant t \leqslant 3\xi, \end{aligned}$$

where

$$f_0(u) = \sum_{i=1}^{k} \gamma_{i00} \exp(-\lambda_i u), \tag{2.24}$$

$$f_1(u) = \sum_{i=1}^{k} (\gamma_{i10} + \gamma_{i11}u) \exp(-\lambda_i u),$$

and the constants γ_{imr} are given by

$$\gamma_{imr} = \phi_{\mathcal{A}} \boldsymbol{C}_{imr} \boldsymbol{Q}_{\mathcal{AF}} \exp(\boldsymbol{Q}_{\mathcal{FF}}\xi)\boldsymbol{u}_{\mathcal{F}}.$$

We use the first two intervals, $0 < u < \xi$ and $\xi < u < 2\xi$, in (2.21) because, from (2.13) and (2.14), the functions $f_{T_0}(t)$ and ${}^{e}\boldsymbol{G}_{\mathcal{AF}}(t)$ depend on ${}^{\mathcal{A}}\boldsymbol{R}(t-\xi)$. Hawkes *et al.* (1990) do give further exact results for $u > 2\xi$, and hence $t > 3\xi$, but the following approximation is preferred.

Approximate evaluation of the survivor function ${}^{\mathcal{A}}\boldsymbol{R}(u)$ for large u

For values of apparent open time $t > 3\xi$ we shall use, in place of the exact solution, an asymptotic form which has been found in practice to be very accurate (even for smaller values of t than this, in some cases). From equation (2.18) we see that the asymptotic behaviour of ${}^{\mathcal{A}}\boldsymbol{R}(u)$ depends on the values of s which render singular the matrix $\boldsymbol{W}(s)$ defined as

$$\boldsymbol{W}(s) = s\boldsymbol{I} - \boldsymbol{H}(s), \tag{2.25}$$

where

$$\boldsymbol{H}(s) = \boldsymbol{Q}_{\mathcal{AA}} + \boldsymbol{Q}_{\mathcal{AF}}(s\boldsymbol{I} - \boldsymbol{Q}_{\mathcal{FF}})^{-1}[\boldsymbol{I} - \exp(-(s\boldsymbol{I} - \boldsymbol{Q}_{\mathcal{FF}})\xi]\boldsymbol{Q}_{\mathcal{FA}}, \tag{2.26}$$

provided s is not an eigenvalue of $\boldsymbol{Q}_{\mathcal{FF}}$ so that $(s\boldsymbol{I} - \boldsymbol{Q}_{\mathcal{FF}})^{-1}$ exists. In other words, we are interested in the roots of the determinantal equation

$$\det[\boldsymbol{W}(s)] = 0. \tag{2.27}$$

Models of ion channels are normally assumed to obey the principle of microscopic reversibility, in the absence of external energy supply (see Colquhoun & Hawkes 1982, pp. 24–25). Under these conditions, Jalali & Hawkes (1992*b*) proved that $\det \boldsymbol{W}(s) = 0$ has exactly $k_{\mathcal{A}}$ real roots, denoted s_i. If these are distinct, then, as $u \to \infty$,

$${}^{\mathcal{A}}\boldsymbol{R}(u) \sim \sum_{i=1}^{k_{\mathcal{A}}} {}^{\mathcal{A}}\boldsymbol{R}_i \mathrm{e}^{-u/\tau_i}, \tag{2.28}$$

where

$$\tau_i = -1/s_i, \quad {}^{\mathcal{A}}\boldsymbol{R}_i = \boldsymbol{c}_i\boldsymbol{r}_i/\boldsymbol{r}_i\boldsymbol{W}'(s_i)\boldsymbol{c}_i, \tag{2.29}$$

and $\boldsymbol{c}_i$, $\boldsymbol{r}_i$ are the right and left (column and row) eigenvectors of $\boldsymbol{H}(s_i)$ corresponding to the root s_i, which is also an eigenvalue of $\boldsymbol{H}(s_i)$.

The matrix derivative in the above results can be evaluated as

$$\boldsymbol{W}'(s) = \boldsymbol{I} + \boldsymbol{Q}_{\mathcal{AF}}[\boldsymbol{S}^*_{\mathcal{FF}}(s)(s\boldsymbol{I} - \boldsymbol{Q}_{\mathcal{FF}})^{-1} - \xi(\boldsymbol{I} - \boldsymbol{S}^*_{\mathcal{FF}}(s))]\boldsymbol{G}^*_{\mathcal{FA}}(s), \tag{2.30}$$

where $\boldsymbol{S}^*_{\mathcal{FF}}(s)$ and $\boldsymbol{G}^*_{\mathcal{FA}}(s)$ are defined in equations (2.17) and (2.4), respectively.

In the special case where there is only one open state and one shut state, Jalali & Hawkes (1992*a*) showed that there will also be infinitely many complex conjugate pairs of roots. However, this is of no interest for the purposes of this paper.

One consequence of these results is that, from quation (2.14), we can represent the asymptotic probability density of the e-open times in the form

$$f_{T_0}(t) \approx \sum_{i=1}^{k_{\mathcal{A}}} a_i(1/\tau_i)\exp(-(t-\xi)/\tau_i), \tag{2.31}$$

where the constants, a_i, are given by

$$a_i = \tau_i\phi_{\mathcal{A}}\, {}^{\mathcal{A}}\boldsymbol{R}_i \boldsymbol{Q}_{\mathcal{AF}} \exp(\boldsymbol{Q}_{\mathcal{FF}}\xi)\boldsymbol{u}_{\mathcal{F}}. \tag{2.32}$$

It should be noted that this approximation refers to large t, not the whole distribution from $t = \xi \to \infty$. However, if you do think of it as applying over the whole range from $t = \xi \to \infty$, the density being zero for $0 < t < \xi$, the a_i can be thought of as areas. There is no reason why they should add up to exactly 1, though they are usually quite close (see examples in Hawkes *et al.* (1992) and table 1 here). The beauty of this result is that it has exactly the same form, a mixture of $k_{\mathcal{A}}$ exponentials, that is found in the case of perfect resolution (to which it reduces when $\xi = 0$). The time constants, τ_i for the exponentials are, of course, different when brief events are missed.

Similar results can be obtained for apparent shut times, by simply interchanging the roles of $\mathcal{A}$ and $\mathcal{F}$ in the above equations, to obtain ${}^{\mathcal{F}}\boldsymbol{R}(u)$ and hence ${}^{e}\boldsymbol{G}_{\mathcal{FA}}(t)$ and the corresponding probability density $f_{T_c}(t)$.

Comparing observed and theoretical distributions: modified areas

It should be emphasized that the form (2.31) applies for 'large' t and does not, in general, give good results for $t < 3\xi$ (although sometimes it does). It has, however, got the merit that it is a mixture of exponential distributions, and so can be compared directly with the ideal ($\xi = 0$) distribution, or with the multiexponential distribution which would commonly be fitted to experimental results (see Colquhoun & Sigworth 1995). Such comparisons, strictly speaking, are not necessary, because the exact distribution (e.g. equation (2.14) for observed open times) can be fitted, or, better, the whole of the data fitted simultaneously, as discussed in § 5. Nevertheless, this sort of comparison is often convenient. The time constants can be compared directly, but the areas associated with each time constant can not, because the asymptotic distribution in (2.31), like the exact distribution, has zero probability for $t < \xi$. Before the comparison can be made, the asymptotic distribution must be projected back to $t = 0$, and this is achieved by assuming that equation (2.31) applies for all $t > 0$ and rescaling it so that the total area is unity. The adjusted distribution of apparent open times then takes the form

$$f_{T_0}(t) = \sum_{i=1}^{k_{\mathcal{A}}} a_i'(1/\tau_i)\exp(-t/\tau_i), \quad t \geqslant 0. \tag{2.33}$$

The factors e^{ξ/τ_i} have been incorporated into modified areas, a_i', defined from $t = 0 \to \infty$ as

$$a_i' = a_i e^{\xi/\tau_i} / \Sigma a_i e^{\xi/\tau_i}, \tag{2.34}$$

where a_i were defined in (2.32), and the denominator serves to normalize the modified areas so that they add up to unity exactly.

Such an adjustment, in effect, is an attempt to correct for missing short open times (but it does not correct for the effect on apparent observed open times of missing short shut times). Examples of the use of such modified areas are given in § 6 (see also Hawkes *et al.* 1992).

3. Joint and conditional probability densities for adjacent intervals

A number of authors have considered the correlation between successive open or shut intervals, with or without allowing for time interval omission (see, for example, Fredkin *et al.* 1985; Colquhoun & Hawkes 1977; Ball & Sansom 1988*b*; Ball *et al.* 1988). However, correlation coefficients tend to be relatively uninformative, and

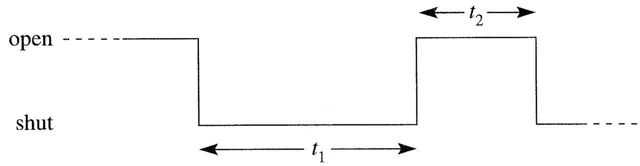


Figure 2. Illustration of consecutive apparent sojourns. A shut sojourn of duration t_1 is followed by an open sojourn of duration t_2.

restricted in the range of values which can be achieved, when the marginal distributions have an exponential form (see, for example, Downton 1970). The full joint distributions or conditional distributions will usually be more useful (see § 1).

Consider two consecutive intervals, for example, a shut time of duration t_1 followed an open time of duration t_2, as illustrated in figure 2.

The joint distribution of an apparent shut time (e-shut time), T_c, and the apparent open time, T_0, which immediately follows it, is given by

$$f_{T_\text{c},T_0}(t_1, t_2) = \phi_{\mathcal{F}}\, {}^{e}\boldsymbol{G}_{\mathcal{F}\mathcal{A}}(t_1)\, {}^{e}\boldsymbol{G}_{\mathcal{A}\mathcal{F}}(t_2)\boldsymbol{u}_{\mathcal{F}}. \tag{3.1}$$

Dependence of open time on preceding shut time

A convenient way to study the association between the two random variables without having to use three-dimensional graphs is to use conditional densities; for example, the distribution of the apparent open time, conditional on the apparent shut time that precedes it, is given by

$$f_{T_0|T_\text{c}}(t_2|t_1) = f_{T_\text{c},T_0}(t_1, t_2)/f_{T_\text{c}}(t_1). \tag{3.2}$$

These distributions are easily computed from any specified mechanism (i.e. from the $\boldsymbol{Q}$ matrix) by means of the results given in the previous section. The exact forms of ${}^{\mathcal{F}}\boldsymbol{R}(u)$ and ${}^{\mathcal{A}}\boldsymbol{R}(u)$ should be used for small values of u (in practice it has been found adequate to use the exact form for u less than 2ξ), and the asymptotic forms for larger values. The result in (3.2) implies that, just as in the ideal case, the conditional distribution has exactly the same $k_{\mathcal{A}}$ time constants, τ_i, for the asymptotic forms (as found in equation (2.29)) as for the unconditional density (equation (2.31)). The dependence of open time on adjacent shut time is manifested in the different areas for each of these components in the conditional and unconditional distributions. For the conditional distribution, (3.2), the areas for the $k_{\mathcal{A}}$ exponential components of the asymptotic form (still given by (2.31)) are given by

$$a_i = \tau_i \phi_{\mathcal{F}}\, {}^{e}\boldsymbol{G}_{\mathcal{F}\mathcal{A}}(t_1)\, {}^{\mathcal{A}}\boldsymbol{R}_i \boldsymbol{Q}_{\mathcal{A}\mathcal{F}} \exp(\boldsymbol{Q}_{\mathcal{F}\mathcal{F}}\xi)\boldsymbol{u}_{\mathcal{F}}/f_{T_\text{c}}(t_1). \tag{3.3}$$

These areas depend, of course, on the length, t_1, of the preceding shut time on which the distribution is conditioned. Modified areas can, if required, be obtained in exactly the same way as for the unconditional distribution (see the discussion at the end of § 2).

The predicted agreement between the time constants of the conditional and unconditional distributions was observed in analyses of experimental data by Blatz & Magleby (1989), Weiss & Magleby (1989), McManus & Magleby (1989) and Gibb & Colquhoun (1992). These authors show that the shape of the conditional distribution depends only on changes in the area associated with each time constant, as predicted by the theory given here; they plot the area of each component against t_1, which is a useful way to represent the dependence. Examples of such plots are given by Srodzinski (1994).

Mean of the conditional distribution

The mean apparent open time, for open times that follow an apparent shut time of length t_1, is defined as

$$E(T_0|T_c = t_1) = \int_0^\infty t_2 f_{T_0|T_c}(t_2|t_1)\,dt_2. \tag{3.4}$$

A plot of this mean open time against t_1 is a useful way to represent the dependence (see figure 9).

The mean can be found by differentiating the Laplace transform of (3.2), changing the sign and setting $s = 0$. This gives an (exact) expression for the mean as follows:

$$E(T_0|T_c = t_1) = \xi + \phi_{\mathcal{F}}\, {}^{e}\boldsymbol{G}_{\mathcal{FA}}(t_1)\left[-\frac{d}{ds}{}^{\mathcal{A}}\boldsymbol{R}^*(s)\right]_{s=0}\boldsymbol{Q}_{\mathcal{AF}}\exp(\boldsymbol{Q}_{\mathcal{FF}}\xi)\boldsymbol{u}_{\mathcal{F}}/f_{T_c}(t_1), \tag{3.5}$$

where

$$\left.\begin{aligned}
&\left[-\frac{d}{ds}{}^{\mathcal{A}}\boldsymbol{R}^*(s)\right]_{s=0} = \boldsymbol{V}_{\mathcal{A}}^{-1}\boldsymbol{Q}_{\mathcal{AA}}^{-2} - \boldsymbol{V}_{\mathcal{A}}^{-1}\left[\frac{d}{ds}\boldsymbol{V}_{\mathcal{A}}^*(s)\right]_{s=0}\boldsymbol{V}_{\mathcal{A}}^{-1}\boldsymbol{Q}_{\mathcal{AA}}^{-1},\\
&\text{in which}\\
&\boldsymbol{V}_{\mathcal{A}}^*(s) = \boldsymbol{I} - \boldsymbol{G}_{\mathcal{AF}}^*(s)\boldsymbol{S}_{\mathcal{FF}}^*(s)\boldsymbol{G}_{\mathcal{FA}}^*(s),\\
&\text{and}\\
&\left[\frac{d}{ds}\boldsymbol{V}_{\mathcal{A}}^*(s)\right]_{s=0} = -\boldsymbol{Q}_{\mathcal{AA}}^{-1}\boldsymbol{G}_{\mathcal{AF}}\boldsymbol{S}_{\mathcal{FF}}\boldsymbol{G}_{\mathcal{FA}} - \boldsymbol{G}_{\mathcal{AF}}\boldsymbol{S}_{\mathcal{FF}}\boldsymbol{Q}_{\mathcal{FF}}^{-1}\boldsymbol{G}_{\mathcal{FA}}\\
&\qquad\qquad -\xi\boldsymbol{G}_{\mathcal{AF}}\exp(\boldsymbol{Q}_{\mathcal{FF}}\xi)\boldsymbol{G}_{\mathcal{FA}}.
\end{aligned}\right\} \tag{3.6}$$

A similar expression will hold for

$$\left[-\frac{d}{ds}{}^{\mathcal{F}}\boldsymbol{R}^*(s)\right]_{s=0}.$$

Initial vector for an e-opening

The dependence of the e-open time on the duration of the previous e-shut time results entirely from the fact that the entry probabilities (or 'initial vector') of the e-opening, i.e. the probabilities of occupying the various open states at the instant the e-open period begins, depend on the duration of the previous e-shut interval. These entry probabilities for each open state, given that the duration of the preceding e-shut sojourn was t_1, will be given by the vector

$$\phi_{\mathcal{A}|T_c=t_1} = \phi_{\mathcal{F}}\, {}^{e}\boldsymbol{G}_{\mathcal{FA}}(t_1)/f_{T_c}(t_1). \tag{3.7}$$

This will be different from the *unconditional* (equilibrium) entry probability vector $\phi_{\mathcal{A}}$ (see equation (2.10)); the difference will be illustrated by numerical examples given in § 6.

Dependence of shut time on following open time

Of course one could equally well obtain the distribution of the apparent shut-time conditional on the length of the opening that *follows* it. This sort of distribution was used (see, for example, McManus & Magleby 1989) to check on the reversibility of the mechanisms that underlie experimental observations. The mechanism used for

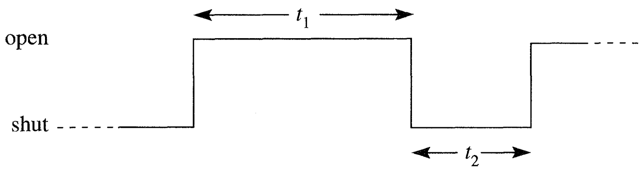


Figure 3. Illustration of consecutive apparent sojourns. An open sojourn of duration t_1 is followed by a shut sojourn of duration t_2.

illustration in § 6 is reversible, but when events are missed it is possible that it could nevertheless appear to be irreversible. Srodzinski (1994) found that there could be small differences between distributions of *apparent* open times that are conditional on preceding shut time and those conditional on following shut time. However, these were restricted to the fourth significant figure in her examples, and would therefore be impossible to detect in experiments, and they are too small to be seen on the graphs in figures 8 and 9. No consistent differences between open times that followed or preceded specified shut times could be observed in the experiments of McManus & Magleby (1989).

The conditional density in this case is given by

$$f_{T_c|T_0}(t_1|t_2) = f_{T_c,T_0}(t_1,t_2)/f_{T_0}(t_2). \tag{3.8}$$

In this case the asymptotic distribution has again the form (2.31) but with $k_{\mathcal{F}}$ components, instead of $k_{\mathcal{A}}$, the relative areas of which are given by

$$a_j = \tau_j \phi_{\mathcal{F}}\, {}^{\mathcal{F}}\boldsymbol{R}_j \boldsymbol{Q}_{\mathcal{FA}} \exp(\boldsymbol{Q}_{\mathcal{AA}}\xi)\, {}^{e}\boldsymbol{G}_{\mathcal{AF}}(t_2)\boldsymbol{u}_{\mathcal{F}}/f_{T_0}(t_2), \tag{3.9}$$

where the τ_j are now the time constants, and ${}^{\mathcal{F}}\boldsymbol{R}_j$ the corresponding matrices, for the asymptotic form of ${}^{\mathcal{F}}\boldsymbol{R}(u)$ calculated in a manner analogous to equations (2.28) and (2.29), interchanging $\mathcal{A}$ and $\mathcal{F}$. Modified areas can be obtained in the same way as at the end of § 2.

The mean e-shut time, conditional on the duration of the e-open time which follows it, is given by

$$E(T_c|T_0 = t_2) = \xi + \phi_{\mathcal{F}} \left[-\frac{\mathrm{d}}{\mathrm{d}s}{}^{\mathcal{F}}\boldsymbol{R}^*(s)\right]_{s=0} \boldsymbol{Q}_{\mathcal{FA}} \exp(\boldsymbol{Q}_{\mathcal{AA}}\xi)\, {}^{e}\boldsymbol{G}_{\mathcal{AF}}(t_2)\boldsymbol{u}_{\mathcal{F}}/f_{T_0}(t_2), \tag{3.10}$$

where

$$\left[-\frac{\mathrm{d}}{\mathrm{d}s}{}^{\mathcal{F}}\boldsymbol{R}^*(s)\right]_{s=0}$$

is obtained from a set of equations similar to (3.6), with $\mathcal{A}$ and $\mathcal{F}$ interchanged.

The row vector of entry probabilities at the start of the e-shut period, conditional on the duration of the following e-open period is slightly more complicated than (3.7). It is given by

$$\phi_{\mathcal{F}|T_0=t_2} = [\mathrm{diag}(\phi_{\mathcal{F}})\, {}^{e}\boldsymbol{G}_{\mathcal{FA}}\, {}^{e}\boldsymbol{G}_{\mathcal{AF}}(t_2)\boldsymbol{u}_{\mathcal{F}}]^{\mathrm{T}}/f_{T_0}(t_2). \tag{3.11}$$

Note that 'T' denotes matrix transpose.

Similar results can be obtained for the joint distribution of an apparent open time, T_0, and the apparent shut time, T_c, which immediately follows it, as illustrated in figure 3.

The joint PDF is

$$f_{T_0,T_c}(t_1,t_2) = \phi_{\mathcal{A}}\, {}^{e}\boldsymbol{G}_{\mathcal{AF}}(t_1)\, {}^{e}\boldsymbol{G}_{\mathcal{FA}}(t_2)\boldsymbol{u}_{\mathcal{A}}. \tag{3.12}$$

Dependence of shut time on preceding open time

The conditional density of the shut time given the *preceding* open time is

$$f_{T_c|T_o}(t_2|t_1) = f_{T_o,T_c}(t_1,t_2)/f_{T_o}(t_1). \tag{3.13}$$

The areas for the $k_{\mathcal{F}}$ exponential components of the asymptotic form of this conditional distribution are given by

$$a_i = \tau_i \phi_{\mathcal{A}}\, {}^e\boldsymbol{G}_{\mathcal{AF}}(t_1)\, {}^{\mathcal{F}}\boldsymbol{R}_i \boldsymbol{Q}_{\mathcal{FA}} \exp(\boldsymbol{Q}_{\mathcal{AA}}\xi)\boldsymbol{u}_{\mathcal{A}}/f_{T_o}(t_1), \tag{3.14}$$

where the τ_i are the time constants for the shut-time distribution as discussed following equation (3.9). Modified areas can be obtained in the same way as at the end of § 2.

The mean apparent shut time, following an apparent open time of length t_1, is given by

$$E(T_c|T_o = t_1) = \xi + \phi_{\mathcal{A}}\, {}^e\boldsymbol{G}_{\mathcal{AF}}(t_1) \left[-\frac{\mathrm{d}}{\mathrm{d}s}\, {}^{\mathcal{F}}\boldsymbol{R}^*(s)\right]_{s=0} \boldsymbol{Q}_{\mathcal{FA}} \exp(\boldsymbol{Q}_{\mathcal{AA}}\xi)\boldsymbol{u}_{\mathcal{A}}/f_{T_o}(t_1). \tag{3.15}$$

The entry probabilities for each shut state to begin an e-shut sojourn, given that the length of the preceding e-open time was t_1, will be given by the vector

$$\phi_{\mathcal{F}|T_o=t_1} = \phi_{\mathcal{A}}\, {}^e\boldsymbol{G}_{\mathcal{AF}}(t_1)/f_{T_o}(t_1). \tag{3.16}$$

Dependence of open time on following shut time

Similarly, the conditional density of the e-open time given the following e-shut time is

$$f_{T_o|T_c}(t_1|t_2) = f_{T_o,T_c}(t_1,t_2)/f_{T_c}(t_2). \tag{3.17}$$

For this conditional distribution the areas for the $k_{\mathcal{A}}$ exponential components of the asymptotic form (2.31) are given by

$$a_i = \tau_i \phi_{\mathcal{A}}\, {}^{\mathcal{A}}\boldsymbol{R}_i \boldsymbol{Q}_{\mathcal{AF}} \exp(\boldsymbol{Q}_{\mathcal{FF}}\xi)\, {}^e\boldsymbol{G}_{\mathcal{FA}}(t_2)\boldsymbol{u}_{\mathcal{A}}/f_{T_c}(t_2), \tag{3.18}$$

where τ_i are the time constants for the open-time distribution, as given by (2.29). Modified areas can be obtained in the same way as at the end of § 2.

The mean e-open time, for open times that precede an e-shut time of length t_2, is given by

$$E(T_o|T_c = t_2) = \xi + \phi_{\mathcal{A}} \left[-\frac{\mathrm{d}}{\mathrm{d}s}\, {}^{\mathcal{A}}\boldsymbol{R}^*(s)\right]_{s=0} \boldsymbol{Q}_{\mathcal{AF}} \exp(\boldsymbol{Q}_{\mathcal{FF}}\xi)\, {}^e\boldsymbol{G}_{\mathcal{FA}}(t_2)\boldsymbol{u}_{\mathcal{A}}/f_{T_c}(t_2). \tag{3.19}$$

The row vector of entry probabilities at the start of an e-open period, conditional on the duration of the following e-shut period, is given by

$$\phi_{\mathcal{A}|T_c=t_2} = [\mathrm{diag}(\phi_{\mathcal{A}})\, {}^e\boldsymbol{G}_{\mathcal{AF}}\, {}^e\boldsymbol{G}_{\mathcal{FA}}(t_2)\boldsymbol{u}_{\mathcal{A}}]^{\mathrm{T}}/f_{T_c}(t_2). \tag{3.20}$$

Open–open pairs

It may also be useful to consider the joint distribution of successive apparent open times, separated by a single apparent shut time. The probability density function for this is

$$f_{T_oT_o'}(t_1,t_2) = \phi_{\mathcal{A}}\, {}^e\boldsymbol{G}_{\mathcal{AF}}(t_1)\, {}^e\boldsymbol{G}_{\mathcal{FA}}\, {}^e\boldsymbol{G}_{\mathcal{AF}}(t_2)\boldsymbol{u}_{\mathcal{F}}. \tag{3.21}$$

Other distributions can be formulated in a similar way. For example, it could be of interest to consider the joint density of successive apparent shut times, separated by a single apparent open time, or the joint distributions of intervals separated by several intermediate intervals. However, the dependence falls off fairly rapidly and, more particularly, it does not add any new information. All the information is contained in the joint distribution of neighbouring intervals (Fredkin *et al.* 1985).

Dependency plot

It was suggested by Magleby & Song (1992) that the joint distribution of an open time and the following shut time could be displayed most clearly in a normalized form which they called a *dependency plot.* They define *dependency* as the (normalized) difference between the actual frequency of particular open–shut time pairs, and the frequency that would be expected if openings and shuttings were independent. Define $f_\mathrm{o}(t_\mathrm{o})$ and $f_\mathrm{s}(t_\mathrm{s})$ as the unconditional probability density functions for open times and shut times, respectively, and $f(t_\mathrm{o}, t_\mathrm{s})$ as the two-dimensional distribution. If there were no correlations then the two-dimensional distribution would simply be the product of the separate distributions, $f_\mathrm{o}(t_\mathrm{o})f_\mathrm{s}(t_\mathrm{s})$. Thus dependency, $d(t_\mathrm{o}, t_\mathrm{s})$ is defined as

$$d(t_\mathrm{o}, t_\mathrm{s}) = \frac{f(t_\mathrm{o}, t_\mathrm{s}) - f_\mathrm{o}(t_\mathrm{o})f_\mathrm{s}(t_\mathrm{s})}{f_\mathrm{o}(t_\mathrm{o})f_\mathrm{s}(t_\mathrm{s})}. \tag{3.22}$$

The bivariate distribution, as in (3.1), is given by

$$f(t_\mathrm{o}, t_\mathrm{s}) = \phi_\mathcal{A}\, {}^e\boldsymbol{G}_{\mathcal{AF}}(t_\mathrm{o})\, {}^e\boldsymbol{G}_{\mathcal{FA}}(t_\mathrm{o})\boldsymbol{u}_\mathcal{A}, \tag{3.23}$$

and the two unconditional distributions for open and shut times, respectively, are, as above,

$$f(t_\mathrm{o}) = \phi_\mathcal{A}\, {}^e\boldsymbol{G}_{\mathcal{AF}}(t_\mathrm{o})\boldsymbol{u}_\mathcal{F}, \quad f(t_\mathrm{s}) = \phi_\mathcal{F}\, {}^e\boldsymbol{G}_{\mathcal{FA}}(t_\mathrm{s})\boldsymbol{u}_\mathcal{A}. \tag{3.24}$$

This will be zero for independent intervals, and a value of +0.5 would indicate that there are 50% more observed interval-pairs than would be expected in the case of independent adjacent intervals. An example is shown later in figure 10. A description of how to calculate the plot from experimental values is given by Magleby & Song (1992).

4. Distributions conditional on a range of adjacent interval lengths

Dependence of open time on preceding shut-time range

It is possible to gain a good deal of insight into mechanisms by inspection of the distribution of open times conditional on the adjacent shut time, and of the shape of the plot of mean open time against adjacent shut time. It was shown in the preceding section how these may be calculated from a specified $\boldsymbol{Q}$ matrix. But these quantities are of little or no value for comparison with experimental results. The difficulty is that all the observed values of the preceding shut time, t_1, will be different, so the best one can do is to look at a histogram of e-open time (t_2) values conditional on the preceding shut time, t_1, being in a specified *range*, say t_lo to t_hi (Blatz & Magleby 1989) (see figure 3.1). Thus we need the conditional probability density

$$f_{T_0}(t_2|t_\mathrm{lo} < T_\mathrm{c} < t_\mathrm{hi}) = \left[\int_{t_\mathrm{lo}}^{t_\mathrm{hi}} f_{T_\mathrm{c},T_0}(t_1, t_2)\,\mathrm{d}t_1\right] \Big/ \int_{t_\mathrm{lo}}^{t_\mathrm{hi}} f_{T_\mathrm{c}}(t_1)\,\mathrm{d}t_1\Bigg]. \tag{4.1}$$

In order to evaluate this distribution, we first define ${}^\mathcal{A}\boldsymbol{K}(u)$ as a cumulative version of ${}^{\mathcal{A}}\boldsymbol{R}(u)$, i.e.

$$ {}^{\mathcal{A}}\boldsymbol{K}(u) = \int_0^u {}^{\mathcal{A}}\boldsymbol{R}(\nu)\,\mathrm{d}\nu. $$

An exact solution for this quantity, which, as before, we shall use for $0 < u \leqslant 2\xi$, can be written as

$$ {}^{\mathcal{A}}\boldsymbol{K}(u) = \sum_{i=1}^{k}\sum_{m=0}^{[u/\xi]}\sum_{r=0}^{m}(-1)^m \boldsymbol{C}_{imr}\Gamma_r(u-m\xi;\lambda_i), \tag{4.2} $$

where Γ is essentially an incomplete Gamma function (and is therefore related to a cumulative Poisson distribution) defined as

$$ \left.\begin{aligned} \Gamma_r(u;\lambda_i) = \int_0^u \nu^r \mathrm{e}^{-\lambda_i\nu}\,\mathrm{d}\nu &= \left[1-\sum_{j=0}^{r}\mathrm{e}^{-\lambda_i u}(\lambda_i u)^j/j!\right] r!/\lambda_i^{r+1}, \quad \lambda_i \neq 0, \\ &= u^{r+1}/(r+1), \quad \lambda_i = 0. \end{aligned}\right\} \tag{4.3} $$

In these results, the λ_i are the k eigenvalues of $-\boldsymbol{Q}$ (see 2.19), and the $\boldsymbol{C}_{imr}$ matrices were defined in (2.23). In the summation, $[u/\xi]$ represents the integer part of u/ξ, so for $0 < u < \xi$ the sum is for $m = 0$ only and so involves $\boldsymbol{C}_{i00}$ only, whereas for $\xi < u < 2\xi$ the sum extends over $m = 0, 1$ and involves $\boldsymbol{C}_{i00}$, $\boldsymbol{C}_{i10}$ and $\boldsymbol{C}_{i11}$.

For longer times, $u > 2\xi$, we use the asymptotic approximation for ${}^{\mathcal{A}}\boldsymbol{R}(u)$ (see § 2) to calculate ${}^{\mathcal{A}}\boldsymbol{K}(u)$ as

$$ {}^{\mathcal{A}}\boldsymbol{K}(u) = {}^{\mathcal{A}}\boldsymbol{K}(2\xi) + \int_{2\xi}^{u} {}^{\mathcal{A}}\boldsymbol{R}(\nu)\,\mathrm{d}\nu \approx {}^{\mathcal{A}}\boldsymbol{K}(2\xi) + \sum_{i=1}^{k_{\mathcal{A}}} {}^{\mathcal{A}}\boldsymbol{R}_i\tau_i(\mathrm{e}^{-2\xi/\tau_i} - \mathrm{e}^{-u/\tau_i}), \quad u > 2\xi, \tag{4.4} $$

where the first term, ${}^{\mathcal{A}}\boldsymbol{K}(2\xi)$, represents the value of ${}^{\mathcal{A}}\boldsymbol{K}(u)$ at $u = 2\xi$ calculated from the exact form given in equations (4.2) and (4.3). The τ_i used here are the asymptotic time constants for the open-time distribution.

As before, exactly similar expressions for ${}^{\mathcal{F}}\boldsymbol{K}(u)$ can be found by interchanging $\mathcal{A}$ and $\mathcal{F}$ in all of these results, and using the appropriate set of time constants τ_i.

Using these results, we easily obtain the unconditional cumulative distributions of e-open and e-shut times which are, respectively. for $t > \xi$,

$$ \left.\begin{aligned} F_{T_0}(t) &= \phi_{\mathcal{A}}\,{}^{\mathcal{A}}\boldsymbol{K}(t-\xi)\boldsymbol{Q}_{\mathcal{AF}}\exp(\boldsymbol{Q}_{\mathcal{FF}}\xi)\boldsymbol{u}_{\mathcal{F}}, \\ F_{T_c}(t) &= \phi_{\mathcal{F}}\,{}^{\mathcal{F}}\boldsymbol{K}(t-\xi)\boldsymbol{Q}_{\mathcal{FA}}\exp(\boldsymbol{Q}_{\mathcal{AA}}\xi)\boldsymbol{u}_{\mathcal{A}}. \end{aligned}\right\} \tag{4.5} $$

Of course $F_{T_0}(t) = F_{T_c}(t) = 0$ for all $t < \xi$.

The conditional distribution defined in (4.1) is given by

$$ \begin{aligned} f_{T_0}(t_2|t_{\mathrm{lo}} < T_{\mathrm{c}} < t_{\mathrm{hi}}) = \phi_{\mathcal{F}}\{{}^{\mathcal{F}}\boldsymbol{K}(t_{\mathrm{hi}}-\xi) - {}^{\mathcal{F}}\boldsymbol{K}(t_{\mathrm{lo}}-\xi)\}\boldsymbol{Q}_{\mathcal{FA}} \\ \times\exp(\boldsymbol{Q}_{\mathcal{AA}}\xi)\,{}^{e}\boldsymbol{G}_{\mathcal{AF}}(t_2)\boldsymbol{u}_{\mathcal{F}}/[F_{T_c}(t_{\mathrm{hi}}) - F_{T_c}(t_{\mathrm{lo}})]. \end{aligned} \tag{4.6} $$

This can be evaluated using the exact forms for ${}^{e}\boldsymbol{G}_{\mathcal{AF}}(t_2)$ when $t_2 < 3\xi$ and the asymptotic form for larger times. The time constants, τ_i, for the $k_{\mathcal{A}}$ exponential components of the asymptotic form (2.31) for large t_2 will, as before, be the same as those for the unconditional distribution of e-open times (see § 2). The areas for each of these components will, however, depend on the range, t_{lo} to t_{hi}, specified for the preceding shut time. These areas will be given by

$$ \begin{aligned} a_i = \phi_{\mathcal{F}}\{{}^{\mathcal{F}}\boldsymbol{K}(t_{\mathrm{hi}}-\xi) - {}^{\mathcal{F}}\boldsymbol{K}(t_{\mathrm{lo}}-\xi)\}\boldsymbol{Q}_{\mathcal{FA}} \\ \times\exp(\boldsymbol{Q}_{\mathcal{AA}}\xi)\tau_i\,{}^{\mathcal{A}}\boldsymbol{R}_i\boldsymbol{Q}_{\mathcal{AF}}\exp(\boldsymbol{Q}_{\mathcal{FF}}\xi)\boldsymbol{u}_{\mathcal{F}}/[F_{T_c}(t_{\mathrm{hi}}) - F_{T_c}(t_{\mathrm{lo}})]. \end{aligned} \tag{4.7} $$

Modified areas can be obtained in the same way as at the end of § 2. The probability that an apparent opening starts in each of the open states (the entry probability), conditional on it being preceded by a shut time in the specified range, is given by the whole $1 \times k_{\mathcal{A}}$ vector which precedes ${}^{e}\boldsymbol{G}_{\mathcal{AF}}(t_2)$ in (4.6), namely

$$\phi_{\mathcal{F}}\{{}^{\mathcal{F}}\boldsymbol{K}(t_{\mathrm{hi}}-\xi) - {}^{\mathcal{F}}\boldsymbol{K}(t_{\mathrm{lo}}-\xi)\}\boldsymbol{Q}_{\mathcal{FA}}\exp(\boldsymbol{Q}_{\mathcal{AA}}\xi)/[F_{T_{\mathrm{c}}}(t_{\mathrm{hi}}) - F_{T_{\mathrm{c}}}(t_{\mathrm{lo}})].$$

A numerical example of these probabilities is given in § 6.

Mean of the conditional distribution

The mean of this distribution is given by

$$E(T_0|t_{\mathrm{lo}} < T_{\mathrm{c}} < t_{\mathrm{hi}}) = \xi + \phi_{\mathcal{F}}\{{}^{\mathcal{F}}\boldsymbol{K}(t_{\mathrm{hi}}-\xi) - {}^{\mathcal{F}}\boldsymbol{K}(t_{\mathrm{lo}}-\xi)\}\boldsymbol{Q}_{\mathcal{FA}}$$
$$\times \exp(\boldsymbol{Q}_{\mathcal{AA}}\xi)\left[-\frac{\mathrm{d}}{\mathrm{d}s}{}^{\mathcal{A}}\boldsymbol{R}^{*}(s)\right]_{s=0}\boldsymbol{Q}_{\mathcal{AF}}\exp(\boldsymbol{Q}_{\mathcal{FF}}\xi)\boldsymbol{u}_{\mathcal{F}}/[F_{T_{\mathrm{c}}}(t_{\mathrm{hi}}) - F_{T_{\mathrm{c}}}(t_{\mathrm{lo}})]. \quad (4.8)$$

For graphical display it will be useful to plot this conditional mean open time against the mean value, over the range t_{lo} to t_{hi}, of the preceding shut time, T_{c}, i.e.

$$E(T_{\mathrm{c}}|t_{\mathrm{lo}} < T_{\mathrm{c}} < t_{\mathrm{hi}}) = \int_{t_{\mathrm{lo}}}^{t_{\mathrm{hi}}} t_1 f_{T_{\mathrm{c}}}(t_1)\,\mathrm{d}t_1/P(t_{\mathrm{lo}} < T_{\mathrm{c}} < t_{\mathrm{hi}})$$
$$= \xi + \phi_{\mathcal{F}}\{{}^{\mathcal{F}}\boldsymbol{M}(t_{\mathrm{hi}}-\xi) - {}^{\mathcal{F}}\boldsymbol{M}(t_{\mathrm{lo}}-\xi)\}\boldsymbol{Q}_{\mathcal{FA}}$$
$$\times \exp(\boldsymbol{Q}_{\mathcal{AA}}\xi)\boldsymbol{u}_{\mathcal{A}}/[F_{T_{\mathrm{c}}}(t_{\mathrm{hi}}) - F_{T_{\mathrm{c}}}(t_{\mathrm{lo}})]. \quad (4.9)$$

In this result ${}^{\mathcal{F}}\boldsymbol{M}(u)$ is defined as

$${}^{\mathcal{F}}\boldsymbol{M}(u) = \int_0^u \nu\ {}^{\mathcal{F}}\boldsymbol{R}(\nu)\,\mathrm{d}\nu.$$

The exact solution for this, used for $0 < u < 2\xi$, is

$${}^{\mathcal{F}}\boldsymbol{M}(u) = \sum_{m=0}^{[u/\xi]}(-1)^m \int_0^u \nu\boldsymbol{N}_m(\nu - m\xi)\,\mathrm{d}\nu$$
$$= \sum_{m=0}^{[u/\xi]}(-1)^m \int_0^{u-m\xi} (\nu + m\xi)\boldsymbol{N}_m(\nu)\,\mathrm{d}\nu$$
$$= \sum_{i=1}^{k}\sum_{m=0}^{[u/\xi]}\sum_{r=0}^{m}(-1)^m\boldsymbol{C}_{imr}[m\xi\varGamma_r(u-m\xi;\lambda_i) + \varGamma_{r+1}(u-m\xi;\lambda_i)]. \quad (4.10)$$

For $u > 2\xi$ the asymptotic form for ${}^{\mathcal{F}}\boldsymbol{R}(u)$ can be used so

$${}^{\mathcal{F}}\boldsymbol{M}(u) \approx {}^{\mathcal{F}}\boldsymbol{M}(2\xi) + \sum_{i=1}^{k_{\mathcal{F}}}{}^{\mathcal{F}}\boldsymbol{R}_i\tau_i[(\tau_i + 2\xi)\mathrm{e}^{-2\xi/\tau_i} - (\tau_i + u)\mathrm{e}^{-u/\tau_i}], \quad (4.11)$$

where the first term represents ${}^{\mathcal{F}}\boldsymbol{M}(u)$ evaluated at $u = 2\xi$ from the exact form given in (4.10). The time constants, τ_i , in (4.11) are as for the unconditional distribution of e-shut times. In (4.10), $\boldsymbol{N}_m(u)$ and $\boldsymbol{C}_{imr}$ are as given by (2.22) and (2.23) (for $m = 0$, 1 and $r = 0$, 1), but replacing $\mathcal{A}$ by $\mathcal{F}$. $\varGamma_r(u)$ is defined in (4.3).

Dependence of shut time on following open-time range

By an exactly similar argument, we can obtain the distribution, analogous with that in equation (3.8), for the distribution of the shut time (τ_1) conditional on the following open time being in the range t_{lo} to t_{hi}. The result is

$$f_{T_c}(t_1|t_{\mathrm{lo}} < T_0 < t_{\mathrm{hi}}) = \phi_{\mathcal{F}}\, {}^{e}\boldsymbol{G}_{\mathcal{FA}}(t_1)\{{}^{\mathcal{A}}\boldsymbol{K}(t_{\mathrm{hi}} - \xi) - {}^{\mathcal{A}}\boldsymbol{K}(t_{\mathrm{lo}} - \xi)\}\boldsymbol{Q}_{\mathcal{AF}} \times \exp(\boldsymbol{Q}_{\mathcal{FF}}\xi)\boldsymbol{u}_{\mathcal{F}}/[F_{T_0}(t_{\mathrm{hi}}) - F_{T_0}(t_{\mathrm{lo}})]. \tag{4.12}$$

The areas for the $k_{\mathcal{F}}$ exponential components of the asymptotic distribution, of form (2.31), are

$$a_i = \phi_{\mathcal{F}}\, {}^{\mathcal{F}}\boldsymbol{R}_i \boldsymbol{Q}_{\mathcal{FA}} \exp(\boldsymbol{Q}_{\mathcal{AA}}\xi)\tau_i\{{}^{\mathcal{A}}\boldsymbol{K}(t_{\mathrm{hi}} - \xi) - {}^{\mathcal{A}}\boldsymbol{K}(t_{\mathrm{lo}} - \xi)\}\boldsymbol{Q}_{\mathcal{AF}} \times \exp(\boldsymbol{Q}_{\mathcal{FF}}\xi)\boldsymbol{u}_{\mathcal{F}}/[F_{T_0}(t_{\mathrm{hi}}) - F_{T_0}(t_{\mathrm{lo}})], \tag{4.13}$$

where the τ_i are the same as the asymptotic time constants for the unconditional distribution of e-shut times (see § 2). Modified areas can be obtained in the same way as at the end of § 2.

Mean of the conditional distribution

The mean of this distribution is obtained similarly to the preceding example, and is

$$E(T_c|t_{\mathrm{lo}} < T_0 < t_{\mathrm{hi}}) = \xi + \phi_{\mathcal{F}}\left[-\frac{\mathrm{d}}{\mathrm{d}s}\, {}^{\mathcal{F}}\boldsymbol{R}^*(s)\right]_{s=0} \boldsymbol{Q}_{\mathcal{FA}} \times \exp(\boldsymbol{Q}_{\mathcal{AA}}\xi)\{{}^{\mathcal{A}}\boldsymbol{K}(t_{\mathrm{hi}} - \xi) - {}^{\mathcal{A}}\boldsymbol{K}(t_{\mathrm{lo}} - \xi)\}\boldsymbol{Q}_{\mathcal{AF}} \times \exp(\boldsymbol{Q}_{\mathcal{FF}}\xi)\boldsymbol{u}_{\mathcal{F}}/[F_{T_0}(t_{\mathrm{hi}}) - F_{T_0}(t_{\mathrm{lo}})]. \tag{4.14}$$

For graphical display it will be useful to plot this conditional mean shut time against the mean value of the following open time, T_0, over the range t_{lo} to t_{hi}, i.e.

$$\begin{aligned} E(T_0|t_{\mathrm{lo}} < T_0 < t_{\mathrm{hi}}) &= \int_{t_{\mathrm{lo}}}^{t_{\mathrm{hi}}} t_2 f_{T_0}(t_2)\,\mathrm{d}t_2/P(t_{\mathrm{lo}} < T_0 < t_{\mathrm{hi}}) \\ &= \xi + \phi_{\mathcal{A}}\{{}^{\mathcal{A}}\boldsymbol{M}(t_{\mathrm{hi}} - \xi) - {}^{\mathcal{A}}\boldsymbol{M}(t_{\mathrm{lo}} - \xi)\}\boldsymbol{Q}_{\mathcal{AF}} \\ &\quad \times \exp(\boldsymbol{Q}_{\mathcal{FF}}\xi)\boldsymbol{u}_{\mathcal{F}}/[F_{T_0}(t_{\mathrm{hi}}) - F_{T_0}(t_{\mathrm{lo}})]. \end{aligned} \tag{4.15}$$

In this result ${}^{\mathcal{A}}\boldsymbol{M}(u)$ is defined by direct analogy with the previous definition of ${}^{\mathcal{F}}\boldsymbol{M}(u)$ given above, using the asymptotic time constants for open times.

Dependence of shut time on preceding open-time range

As in § 3, we can carry out similar calculations to those above for the case when a shut time follows an open time. For the sake of completeness, we list the results without further explanation.

Thus we need the conditional probability density

$$\begin{aligned} f_{T_c}(t_2|t_{\mathrm{lo}} < T_0 < t_{\mathrm{hi}}) &= \left[\int_{t_{\mathrm{lo}}}^{t_{\mathrm{hi}}} f_{T_0,T_c}(t_1, t_2)\,\mathrm{d}t_1\right] \Big/ \left[\int_{t_{\mathrm{lo}}}^{t_{\mathrm{hi}}} f_{T_0}(t_1)\,\mathrm{d}t_1\right] \\ &= \phi_{\mathcal{A}}\{{}^{\mathcal{A}}\boldsymbol{K}(t_{\mathrm{hi}} - \xi) - {}^{\mathcal{A}}\boldsymbol{K}(t_{\mathrm{lo}} - \xi)\}\boldsymbol{Q}_{\mathcal{AF}} \\ &\quad \times \exp(\boldsymbol{Q}_{\mathcal{FF}}\xi)\, {}^{e}\boldsymbol{G}_{\mathcal{FA}}(t_2)\boldsymbol{u}_{\mathcal{A}}/[F_{T_0}(t_{\mathrm{hi}}) - F_{T_0}(t_{\mathrm{lo}})]. \end{aligned} \tag{4.16}$$

This can be evaluated using the exact forms for ${}^{e}\boldsymbol{G}_{\mathcal{FA}}(t_2)$ when $t_2 < 3\xi$ and the asymptotic form for larger times. The time constants, τ_i, for the $k_{\mathcal{F}}$ exponential components of the asymptotic form (2.31) for large t_2 will, as before, be the same as those for other distributions of e-shut times. The areas for each of these components will be given by

$$a_i = \phi_{\mathcal{A}}\{{}^{\mathcal{A}}\boldsymbol{K}(t_{\mathrm{hi}} - \xi) - {}^{\mathcal{A}}\boldsymbol{K}(t_{\mathrm{lo}} - \xi)\}\boldsymbol{Q}_{\mathcal{AF}} \times \exp(\boldsymbol{Q}_{\mathcal{FF}}\xi)\tau_i\, {}^{\mathcal{F}}\boldsymbol{R}_i \boldsymbol{Q}_{\mathcal{FA}} \exp(\boldsymbol{Q}_{\mathcal{AA}}\xi)\boldsymbol{u}_{\mathcal{A}}/[F_{T_0}(t_{\mathrm{hi}}) - F_{T_0}(t_{\mathrm{lo}})]. \quad (4.17)$$

Modified areas can be obtained in the same way as at the end of § 2.

The mean of this distribution is given by

$$E(T_{\mathrm{c}}|t_{\mathrm{lo}} < T_0 < t_{\mathrm{hi}}) = \xi + \phi_{\mathcal{A}}\{{}^{\mathcal{A}}\boldsymbol{K}(t_{\mathrm{hi}} - \xi) - {}^{\mathcal{A}}\boldsymbol{K}(t_{\mathrm{lo}} - \xi)\}\boldsymbol{Q}_{\mathcal{AF}} \times \exp(\boldsymbol{Q}_{\mathcal{FF}}\xi)\left[-\frac{\mathrm{d}}{\mathrm{d}s}{}^{\mathcal{F}}\boldsymbol{R}^*(s)\right]_{s=0}\boldsymbol{Q}_{\mathcal{FA}} \times \exp(\boldsymbol{Q}_{\mathcal{AA}}\xi)\boldsymbol{u}_{\mathcal{A}}/[F_{T_0}(t_{\mathrm{hi}}) - F_{T_{\mathrm{c}}}(t_{\mathrm{lo}})]. \quad (4.18)$$

It will be useful to plot this conditional mean shut time against the mean value, over the range t_{lo} to t_{hi}, of the preceding open time, i.e. $E(T_0|t_{\mathrm{lo}} < T_0 < t_{\mathrm{hi}})$ as given in equation (4.15).

Dependence of open time on following shut-time range

Similarly, we can obtain the conditional density

$$f_{T_0}(t_1|t_{\mathrm{lo}} < T_{\mathrm{c}} < t_{\mathrm{hi}}) = \phi_{\mathcal{A}}\, {}^{e}\boldsymbol{G}_{\mathcal{AF}}(t_1)\{{}^{\mathcal{F}}\boldsymbol{K}(t_{\mathrm{hi}} - \xi) - {}^{\mathcal{F}}\boldsymbol{K}(t_{\mathrm{lo}} - \xi)\}\boldsymbol{Q}_{\mathcal{FA}} \times \exp(\boldsymbol{Q}_{\mathcal{AA}}\xi)\boldsymbol{u}_{\mathcal{A}}/[F_{T_{\mathrm{c}}}(t_{\mathrm{hi}}) - F_{T_{\mathrm{c}}}(t_{\mathrm{lo}})]. \quad (4.19)$$

The areas for the $k_{\mathcal{A}}$ exponential components of the asymptotic distribution, of form (2.31), are

$$a_i = \phi_{\mathcal{A}}\, {}^{\mathcal{A}}\boldsymbol{R}_i \boldsymbol{Q}_{\mathcal{AF}} \exp(\boldsymbol{Q}_{\mathcal{FF}}\xi)\tau_i\{{}^{\mathcal{F}}\boldsymbol{K}(t_{\mathrm{hi}} - \xi) - {}^{\mathcal{F}}\boldsymbol{K}(t_{\mathrm{lo}} - \xi)\}\boldsymbol{Q}_{\mathcal{FA}} \times \exp(\boldsymbol{Q}_{\mathcal{AA}}\xi)\boldsymbol{u}_{\mathcal{F}}/[F_{T_{\mathrm{c}}}(t_{\mathrm{hi}}) - F_{T_{\mathrm{c}}}(t_{\mathrm{lo}})], \quad (4.20)$$

where the τ_i are the same as the asymptotic time constants for other distributions of e-open times. Modified areas can be obtained in the same way as at the end of § 2.

The mean of this distribution is

$$E(T_0|t_{\mathrm{lo}} < T_{\mathrm{c}} < t_{\mathrm{hi}}) = \xi + \phi_{\mathcal{A}}\left[-\frac{\mathrm{d}}{\mathrm{d}s}{}^{\mathcal{A}}\boldsymbol{R}^*(s)\right]_{s=0}\boldsymbol{Q}_{\mathcal{AF}} \times \exp(\boldsymbol{Q}_{\mathcal{FF}}\xi)\{{}^{\mathcal{F}}\boldsymbol{K}(t_{\mathrm{hi}} - \xi) - {}^{\mathcal{F}}\boldsymbol{K}(t_{\mathrm{lo}} - \xi)\}\boldsymbol{Q}_{\mathcal{FA}} \times \exp(\boldsymbol{Q}_{\mathcal{AA}}\xi)\boldsymbol{u}_{\mathcal{A}}/[F_{T_{\mathrm{c}}}(t_{\mathrm{hi}}) - F_{T_{\mathrm{c}}}(t_{\mathrm{lo}})]. \quad (4.21)$$

This may usefully be plotted against the mean value of the following shut time, T_{c}, over the range t_{lo} to t_{hi}, i.e. $E(T_{\mathrm{c}}|t_{\mathrm{lo}} < T_{\mathrm{c}} < t_{\mathrm{hi}})$ as given by equation (4.9).

5. Maximum likelihood fitting

Conventional fitting

Up to now, the fitting of a kinetic model to experimental results has usually been done in a rather haphazard way. For example, Colquhoun & Sakmann (1985) fitted separately the distributions of observed open times, shut times, burst lengths, number of openings per burst and total open time per burst. Approximate corrections for missed events were made retrospectively, and an attempt made to find a mechanism, and the rates for the mechanism, that would predict with reasonable accuracy the various types of observation. There are several problems with this sort of approach.

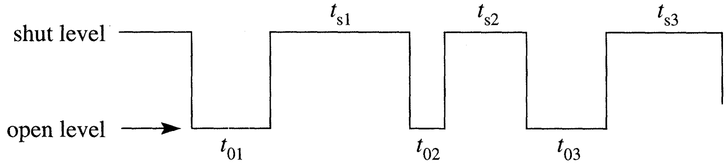


Figure 4. A sequence of alternating apparent open sojourns and apparent shut sojourns.

First, the different types of distribution contain overlapping types of information, and no way is known to combine the information from each type. Second, the correction for missed events is, at best, approximate. And third, the analysis ignores entirely the information from correlations between open and shut times. The idea of fitting a mechanism directly to an entire sequence of open and shut times was first proposed by Horn & Lange (1983). At that time there were no good corrections for missed events, so the likelihood of the sequence could not be computed accurately. More recently Sine *et al.* (1990) used an approximation to the distribution of observed results to calculate the likelihood of a sequence of openings and shuttings, and used this for fitting.

Suppose that we have a series of observed open and shut times which alternate as shown in figure 4. The conventional maximum likelihood fit of the apparent open times (see Colquhoun & Sigworth, 1995) would be done as follows. For open times we have observed values of $t_{o1}, t_{o2}, t_{o3}, \ldots$. The parameters to be estimated are the time constants τ_i, and their associated areas a_i, for the conventional multiexponential PDF in (5.2) (these parameters are, of course, mostly related very indirectly to the actual rate constants in the underlying mechanism). The likelihood, l , of a particular set of parameters, i.e. the probability (density) of observing t_{o1} *and* t_{o2} *and* t_{o3} and ... given a particular set of parameters, can be taken as the product of the probability densities for each opening (this is not strictly correct if successive open times are correlated, but it is usually done anyway), i.e.

$$l = f(t_{o1})f(t_{o2})f(t_{o3})\ldots, \tag{5.1}$$

where

$$f(t) = \sum_{i=1}^{k_{\mathcal{A}}} a_i(1/\tau_i)\exp(-t/\tau_i). \tag{5.2}$$

In practice, it is usual to find the log-likelihood, L,

$$L = \log(l), \tag{5.3}$$

by adding the $\log[f(t)]$ values.

In order to fit the mechanism directly to the observations, the parameters to be estimated would not be the time constants and areas of exponentials, but rather they would be the actual rate constants in the mechanism, i.e. the elements of the $\boldsymbol{Q}$ matrix. If, in addition, we allow for missed events, then the probability densities in (5.1) could be found as specified in (2.14), rather than using the form in (5.2). Thus the likelihood to be maximized when fitting the distribution of observed open times would be

$$l = \phi_{\mathcal{A}}\,{}^{e}\boldsymbol{G}_{\mathcal{AF}}(t_{o1})\boldsymbol{u}_{\mathcal{F}} \cdot \phi_{\mathcal{A}}\,{}^{e}\boldsymbol{G}_{\mathcal{AF}}(t_{o2})\boldsymbol{u}_{\mathcal{F}} \cdot \phi_{\mathcal{A}}\,{}^{e}\boldsymbol{G}_{\mathcal{AF}}(t_{o3})\ldots\boldsymbol{u}_{\mathcal{F}}. \tag{5.4}$$

This, however, is unlikely to work well, because the number of parameters will almost certainly be too large to be defined by the open times alone.

Fitting the whole record

It is far better to fit open and shut times simultaneously so information from both is taken into account. In the case where there is only one channel contributing to the observed record this can be done very elegantly now that missed events can be taken into account at the time of fitting (it was not possible when corrections for missed events could be made only retrospectively). We simply calculate the likelihood as the probability (density) of the whole observed sequence, namely

$$l = \phi_{\mathcal{A}}\, {}^{e}\boldsymbol{G}_{\mathcal{AF}}(t_{o1})\, {}^{e}\boldsymbol{G}_{\mathcal{FA}}(t_{s1})\, {}^{e}\boldsymbol{G}_{\mathcal{AF}}(t_{o2})\, {}^{e}\boldsymbol{G}_{\mathcal{FA}}(t_{s2})\, {}^{e}\boldsymbol{G}_{\mathcal{AF}}(t_{o3}) \ldots \boldsymbol{u}_{\mathcal{F}}. \tag{5.5}$$

The parameters in $\boldsymbol{Q}$ are adjusted to maximize this likelihood. This not only takes into account both open and shut times but also takes into account their sequence: if there are correlations between open and shut times, as there are for nicotinic and NMDA receptors, then there will be information in the sequence and this method exploits it. Notice that $\phi_{\mathcal{A}}$ is a $(1 \times k_{\mathcal{A}})$ vector that gives the probability that an opening starts in each of the open states at equilibrium (found as in (2.10)), and the first two factors in (5.5), $\phi_{\mathcal{A}}\, {}^{e}\boldsymbol{G}_{\mathcal{AF}}(t_{o1})$ again form a $(1 \times k_{\mathcal{F}})$ vector that gives the probability that the next shut period starts in each of the shut states following an opening of length t_{o1}. Similarly the first three factors in (5.5), $\phi_{\mathcal{A}}\, {}^{e}\boldsymbol{G}_{\mathcal{AF}}(t_{o1})\, {}^{e}\boldsymbol{G}_{\mathcal{FA}}(t_{s1})$, again form a $(1 \times k_{\mathcal{A}})$ vector that gives the probability that the next opening starts in each of the open states following an opening of length t_{o1} and a shutting of length t_{s1}, and so on, right up to the end of the data record.

Note that, in this approach, no distributions at all are fitted. The likelihood in (5.5) is maximized directly. However, in order to judge how well the estimates so produced can describe the observations, distributions should be displayed after the fitting has been done. The observations would be plotted as histograms in the usual way, and on each histogram would be superimposed the theoretical distribution, calculated from the estimate of $\boldsymbol{Q}$ obtained during the fitting process, as exemplified in § 6. If the fitting was successful there should be good agreement between the histogram and the calculated distribution. The curves may well not fit as well as those obtained by conventional fitting methods, because the calculated distributions are constrained by the fact that they all derive from a common $\boldsymbol{Q}$ matrix. Conventional separate fits to, for example, open times and shut times, lack this constraint (i.e. there is no reason why the separate fits should be compatible with the same mechanism), so the conventional fits may appear to be better.

Estimatability of parameters

Even with the method just described, there will often not be enough information in a single record for reasonable estimates to be made of all the rate constants in the mechanism. This problem may be solved by fitting several different sorts of record simultaneously (see below), but if this is not possible then it may be necessary to fix some of the parameters to values that are either plausible guesses or, better, have been estimated from separate experiments. The number of parameters to be estimated may also be reduced by constraining the ratio of two parameters; for example the association rate constant for a second binding reaction might plausibly be constrained to be half of that for the first binding (see example in § 6).

Simultaneous fits of several data sets

A virtue of the method defined by (5.5) is that it is easy to fit several different data sets simultaneously. For example, single-channel records obtained at several different agonist concentrations may be combined, in order to increase the amount of information that is available about the parameters. Still better, steady-state results, with likelihoods calculated from (5.5), might be combined with non-stationary single-channel data (e.g. concentration jumps or voltage jumps). The relevant theory for single channels after a jump, with allowance for missed events, is given by Merlushkin & Hawkes (1995) and Colquhoun *et al.* (1997). In each case we simply find the log-likelihood, $L = \log(l)$, for each data set, denoted L_1, L_2, L_3, etc., and maximize the overall log-likelihood

$$L = L_1 + L_2 + L_3 + \cdots. \tag{5.6}$$

What to do if the whole record is not from one channel

The method specified in (5.5) can be used when all the open and shut times that are predicted by the model are contained in the observations. However, it is quite common for steady-state single-channel records to be obtained from membrane patches that contain an unknown number of channels. It would be possible to generalize the theory given here for a patch that contained N channels, but it is debatable whether this would be worth while because in most cases N is unknown and cannot be estimated from the data with any accuracy (see, for example, discussion in Colquhoun & Hawkes 1995*a*, p. 431).

The problem of having an unknown number of channels in the patch has usually been solved in the past by concentrating on sections of the record that, almost certainly, originate from one individual channel. For example, Sakmann *et al.* (1980) pointed out that the clusters of openings, often seen at medium and high agonist concentrations are separated by long desensitized periods, and the openings within each cluster almost certainly come from one individual channel. At low agonist concentrations when activations are rare, it is impossible to know whether consecutive openings are from the same channel or not (and therefore impossible to know whether the shut time between the openings can be predicted by the model or not). Nevertheless it is usual that we can say that some sections of the record are all from one channel. For example Colquhoun & Sakmann (1985) exploited the fact that all the (1–10 or so) openings in a burst (single activation) of the nicotinic receptor were almost certainly from the same channel. The shut times within such bursts can be predicted by the model. We can, therefore, use a modified version of the fitting method above if we can define a critical gap length, t_{crit} say, such that we can be virtually sure that all openings separated by gaps shorter than this originate from the same channel. Sequences of openings and shuttings defined in this way will be referred to as *groups*, and all the openings and shuttings *within* a group can be fitted by calculating a likelihood as in (5.5). However, the initial and final vectors used in (5.5), which were appropriate for a record at equilibrium, are no longer appropriate in this case. The shut time that precedes the first opening in a group is not known precisely, *but it is known to be greater than* t_{crit}. The probability that the first opening in a group begins in open state i (state 1 or 2 in example (6.1) in the next section) must take account of this knowledge, rather than supposing (as the equilibrium $\phi_{\mathcal{A}}$ used above does) that it could be any length. Likewise, the shutting that follows the last opening of a group is known to be longer than t_{crit}, though how much longer is unsure. We thus can define a likelihood for the rth group, with n openings in it, as

$$l_r = \phi_b\, {}^{e}\boldsymbol{G}_{\mathcal{AF}}(t_{\text{o}1})\, {}^{e}\boldsymbol{G}_{\mathcal{FA}}(t_{\text{s}1})\, {}^{e}\boldsymbol{G}_{\mathcal{AF}}(t_{\text{o}2})\, {}^{e}\boldsymbol{G}_{\mathcal{FA}}(t_{\text{s}2}) \ldots\, {}^{e}\boldsymbol{G}_{\mathcal{AF}}(t_{\text{o}n})\boldsymbol{e}_{\mathcal{F}}, \tag{5.7}$$

where the initial and final vectors, ϕ_b and $\boldsymbol{e}_{\mathcal{F}}$, are now those appropriate to the specified t_{crit} value. These can be defined, not by the method of defining bursts in terms of a subset of short-lived shut states (as in Colquhoun & Hawkes 1982), but directly from the specified t_{crit} value. Thus the $(k_{\mathcal{F}} \times 1)$ column vector at the end does not have all entries $= 1(\boldsymbol{u}_{\mathcal{F}})$ as in (5.5), but is

$$\boldsymbol{e}_{\mathcal{F}} = \boldsymbol{H}_{\mathcal{FA}} \boldsymbol{u}_{\mathcal{A}}, \tag{5.8}$$

where

$$\boldsymbol{H}_{\mathcal{FA}} = \int_{t_{\text{crit}}}^{\infty} {}^{e}\boldsymbol{G}_{\mathcal{FA}}(t)\, \mathrm{d}t. \tag{5.9}$$

This simply specifies that after the last opening of the group one of the shut states is entered and the channel remains within the shut ($\mathcal{F}$) states for any time from t_{crit} to ∞, before eventually reopening. This result can be evaluated, by use of (2.13), (2.28) and (2.29) (exchanging $\mathcal{A}$ and $\mathcal{F}$) as

$$\boldsymbol{H}_{\mathcal{FA}} = \sum_{i=1}^{k_{\mathcal{F}}} {}^{\mathcal{F}}\boldsymbol{R}_i \boldsymbol{Q}_{\mathcal{FA}} \exp(\boldsymbol{Q}_{\mathcal{AA}}\xi) \tau_i \mathrm{e}^{-(t_{\text{crit}}-\xi)/\tau_i}, \tag{5.10}$$

where the τ_i refer to the time constants for the asymptotic shut time distribution.

The first open interval of a group is also known to follow a shut time of at least t_{crit}. If that previous shut time had started in state i, the initial vector for a group, ϕ_b, could be found by taking the ith row of $\boldsymbol{H}_{\mathcal{FA}}$ and scaling it sum to unity. In practice it usually makes little difference which row is used because, after a long time (and t_{crit} is large), the system is insensitive to initial conditions (all postulated mechanisms are supposed ergodic). If in doubt, a sensible choice would be to average the rows by the entry probability vector $\phi_{\mathcal{F}}$, see (2.11), to give

$$\phi_b = \phi_{\mathcal{F}} \boldsymbol{H}_{\mathcal{FA}} / \phi_{\mathcal{F}} \boldsymbol{H}_{\mathcal{FA}} \boldsymbol{u}_{\mathcal{A}}. \tag{5.11}$$

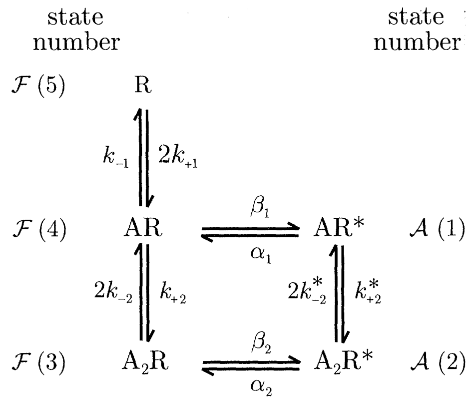
Once t_{crit} is specified the observed record can be divided up in N groups and the log-likelihood for each group, L_i, can be calculated from (5.7). The final log-likelihood, which is to be maximized, is then the sum of the log-likelihoods for each group.

$$L = L_1 + L_2 + L_3 + \cdots + L_N. \tag{5.12}$$

6. Numerical examples

Example of a mechanism

If, for example, there is only one open state or only one shut state, then apparent open and shut times are mutually independent random variables (see Colquhoun & Hawkes (1987) for details). In this case the joint distribution of open and shut times is simply the product of the separate open and shut time densities. In order to illustrate the results given above we therefore need a mechanism with at least two open states and at least two shut states. For the purposes of illustrating the results we shall use a mechanism, discussed in Colquhoun & Hawkes (1982) and Hawkes *et al.* (1992), which has two open and three shut states. Two agonist molecules (A) can bind to the shut (R) conformation, and either singly or doubly occupied channels may open (R*). The scheme is illustrated diagrammatically in (6.1), each state being numbered, and labelled as either shut (set $\mathcal{F}$) or open (set $\mathcal{A}$).



(6.1)

The matrix of rate constants is shown in (6.2):

$$
\boldsymbol{Q} = \begin{array}{c} \\ 1 \\ 2 \\ 3 \\ 4 \\ 5 \end{array}
\begin{array}{c}
\begin{array}{ccccc} \quad 1 \quad & \quad 2 \quad & \quad 3 \quad & \quad 4 \quad & \quad 5 \quad \end{array} \\
\begin{bmatrix}
-(\alpha_1 + k^*_{+2}x_{\mathrm{A}}) & k^*_{+2}x_{\mathrm{A}} & 0 & \alpha_1 & 0 \\
2k^*_{-2} & -(\alpha_2 + 2k^*_{-2}) & \alpha_2 & 0 & 0 \\
0 & \beta_2 & -(\beta_2 + 2k_{-2}) & 2k_{-2} & 0 \\
\beta_1 & 0 & k_{+2}x_{\mathrm{A}} & -(\beta_1 + k_{+2}x_{\mathrm{A}} + k_{-1}) & k_{-1} \\
0 & 0 & 0 & 2k_{+1}x_{\mathrm{A}} & -2k_{+1}x_{\mathrm{A}}
\end{bmatrix}
\end{array}, \tag{6.2}
$$

where x_{A} is the agonist concentration. The particular values for the transition rates that are used for the examples here are shown in (6.3), which is shown partitioned according to whether states are open or shut. The transition rates are all in units of s^{-1}, and correspond to (6.2) with a concentration $x_{\mathrm{A}} = 0.1\ \mu\mathrm{M}$:

$$
\boldsymbol{Q} = \begin{bmatrix} \boldsymbol{Q}_{\mathcal{AA}} & \boldsymbol{Q}_{\mathcal{AF}} \\ \boldsymbol{Q}_{\mathcal{FA}} & \boldsymbol{Q}_{\mathcal{FF}} \end{bmatrix} = \begin{bmatrix}
-3050 & 500 & \vdots & 0 & 3000 & 0 \\
0.666\,667 & -500.666\,667 & \vdots & 500 & 0 & 0 \\
\cdots & \cdots & \cdots & \cdots & \cdots & \cdots \\
0 & 15\,000 & \vdots & -19\,000 & 4000 & 0 \\
15 & 0 & \vdots & 50 & -2065 & 2000 \\
0 & 0 & \vdots & 0 & 10 & -10
\end{bmatrix}. \tag{6.3}
$$

This model is similar to that inferred by Colquhoun & Sakmann (1985) as a description of suberyldicholine-activated ion channels in the frog muscle endplate. Low agonist concentrations were used so the resting state (5) has a long mean lifetime (100 ms) and channel activations are well-separated (by 3789 ms on average). The channel activations occur in bursts which consist predominantly of several 'long' openings separated by brief shuttings. The 'long openings' usually represent a single sojourn in state 2 (A_2R^*) (mean life ≈ 2 ms), since direct transitions between the two open states (1 to 2) are rare. The brief shuttings within a burst consist mainly of single sojourns in state 3 (A_2R, the doubly liganded but shut state with mean life $1/19000 \approx 53\ \mu$s), manifested as the large (73% of area) component of shut times with a time constant of $53\ \mu$s. There are also rare longer shuttings within a burst (as shown by the component of the shut time distribution with time constant 0.485 ms and 0.8% of area in table 1, and figure 5*b*. A few channel activations are brief single openings corresponding mainly of single sojourns in state 1 (mean life = 1/3050 = 0.328 ms), and these are not usually interrupted by brief closures because a channel that returns from state 1 (AR*) to state 4 (AR) is much more likely to lose its agonist molecule and return to the resting state (5) than it is to reopen.

Simulation of observations

Experimental observations were simulated for the purposes of illustrating the theoretical distributions in §§ 1–5, and for testing the direct maximum likelihood fitting method. The transition rates in the $\boldsymbol{Q}$ matrix in (6.3) were used for simulations. A pseudo-random number generator (Wichmann & Hill 1985) was used to produce exponentially distributed lifetimes in each individual state, and to decide which state was entered next. Oscillations within the shut states (3, 4 and 5) were concatenated into a single shut time, and similarly for open times. The effect of limited time resolution was simulated by imposing a fixed time resolution on the results, as described by Colquhoun & Sigworth (1995). For the purpose of illustrating the fit of the relationships described above, 81 920 intervals (40 960 open times and 40 960 shut times) were simulated with a resolution of 1 µs. After imposition of a resolution of 50 µs there were 22249 apparent openings; after imposition of a resolution of 100 µs there were 14 712 apparent openings, and after imposition of a resolution of 200 µs there were 10 049 apparent openings.

The distributions that were described in earlier sections will be illustrated by showing the theoretical values calculated from the equations above, with the $\boldsymbol{Q}$ matrix in (6.3). In cases where it is possible, the result will be superimposed on the corresponding distribution of the simulated observations, to show that the equations do indeed describe the effect of limited time resolution. Note that the calculated distributions in figures 5–8, and the calculated means in figure 9, have not been fitted to the simulated observations, but have been calculated from the parameter values (6.3) that were used for the simulation.

Display of distributions

Table 1 shows the time constants and areas for open-time distributions and shut time distribution. The ideal distribution (no events missed) is shown in the column headed $\xi = 0$. The other columns show the time constants (τ) and areas (a) for the exponential components of the asymptotic distributions of apparent open and shut times with resolutions of $\xi = 50$, 100 and 200 µs, from equations (2.31) and (2.32). The areas projected back to $t = 0$ are also given; they are denoted a' and calculated as in (2.34).

Figure 5 shows the distributions of apparent open time with resolutions of $\xi = 50$, 100 and 200 µs. In each case the ideal distribution ($\xi = 0$, appropriately scaled) is superimposed as a dashed line. The continuous line shows the distribution calculated from (2.14), with the exact form being used for $\xi < t < 3\xi$, and the asymptotic form (as given in table 1) for longer open times. The calculated distribution fits the simulated observations closely in each case. It is clear from figure 5 that the proportion of 'short openings' appears to increase as the resolution gets worse. The reason for this is clear from table 1. The time constant (of the asymptotic approximation) for the faster component, 0.328 ms, is hardly affected by the resolution, because short

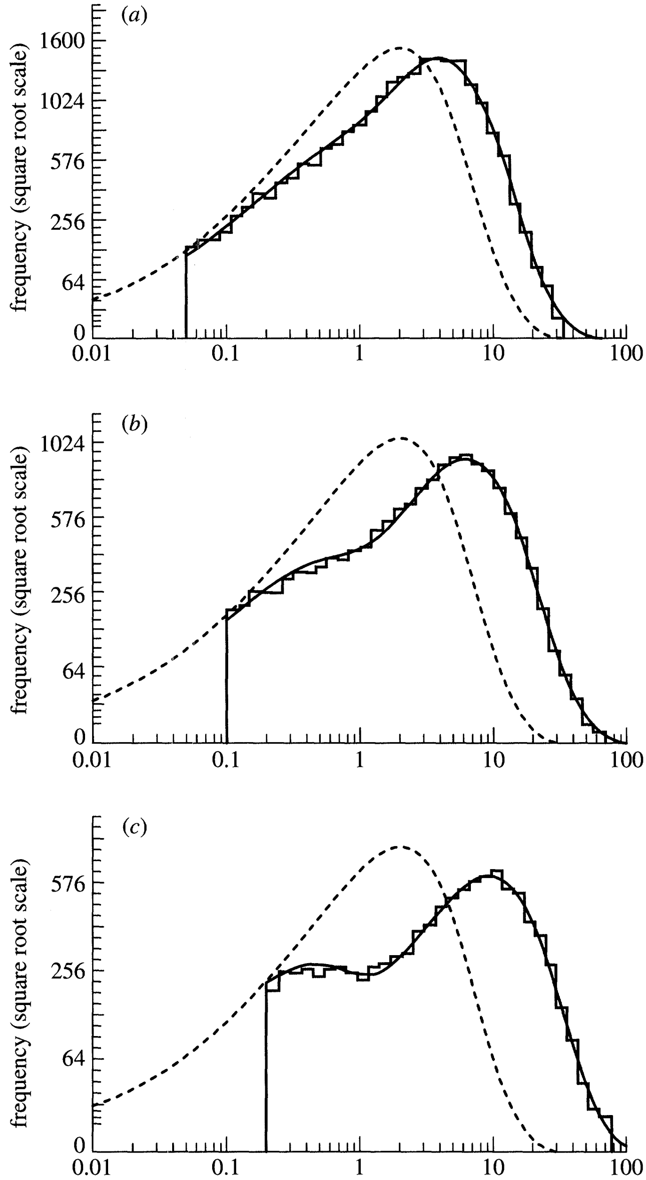


Figure 5. Distribution of apparent open times, with resolutions (ξ) of (a) 50 μs, (b) 100 μs and (c) 200 μs. In this figure, and all subsequent figures, histograms are presented to show the distribution of log (duration) with a square root transformation of the ordinate (McManus *et al.* 1987; Sigworth & Sine 1987). The solid line shows the theoretical distribution, calculated from equations (2.24), (2.31) and (2.32); the exact calculation was used up to $t = 3\xi$, and the asymptotic form was used for larger t. The time constants and areas for the asymptotic form are given in table 1. The histogram shows simulated data, on which the appropriate resolution has been imposed (see text). The dashed line shows the ideal distribution (scaled to have the same area above $t = \xi$) in which no events are missed.

openings are rarely interrupted by short shuttings, so there is nothing to be missed. On the other hand, the time constant for 'long openings' increases from 1.997 ms in

Table 1. *Time constants* (τ, *in* ms) *and areas* (a) *for the ideal* ($\xi = 0$) *distribution of open and shut times, and for the asymptotic distributions of apparent open and shut times with resolutions of* $\xi = 50$, 100 *and* 200 μs

(The areas projected back to $t = 0$, denoted a' and calculated as in (2.34), are also given.)

resolution (μs)		$\xi = 0$	$\xi = 50$	$\xi = 100$	$\xi = 200$
open times	τ_1	0.3279	0.3281	0.3284	0.3289
	a_1	0.0724	0.1163	0.1507	0.1588
	a'_1	0.0724	0.1314	0.1915	0.2532
	τ_2	1.997	3.887	6.138	8.907
	a_2	0.9276	0.8837	0.8492	0.8411
	a'_2	0.9276	0.8686	0.8085	0.7468
shut times	τ_1	0.0526	0.0543	0.0585	0.0791
	a_1	0.7297	0.5152	0.2858	0.0463
	a'_1	0.7297	0.7277	0.6916	0.3798
	τ_2	0.4847	0.4853	0.4859	0.4870
	a_2	0.0084	0.0131	0.0167	0.0176
	a'_2	0.0084	0.0082	0.0090	0.0174
	τ_3	3789	3952	4105	4387
	a_3	0.2619	0.4694	0.6835	0.9196
	a'_3	0.2619	0.2642	0.2994	0.6028

the ideal case to 8.907 ms with a resolution of 200 μs. The 'long openings' are often separated by short closures, most of which are missed with low resolution, so there appear to be fewer, but longer, openings.

The apparent shut-time distribution

Figure 6 shows the distributions of apparent shut times, displayed as for the open times in figure 5. In strong contrast to the results for open times, the distributions are little affected by the resolution (apart from the fact that shut times less than ξ are obviously missing). This, of course, is a result of the fact that there are relatively few short openings, so even with poor resolution, few openings are missed. Inspection of the results for shut times in table 1 reinforces this conclusion. The area, a_i, for the shortest component falls rapidly because of the loss of short shuttings, but when projected back to $t = 0$ (see equation (2.33)) the values of a'_i are seen to change little from the value of 73% seen with perfect resolution, for resolutions of 50 and 100 μs. There is only a slight lengthening of the longest component of shut times as the resolution gets worse, and the intermediate and short components change little down to a resolution of 100 μs. At the really poor resolution of $\xi = 200$ μs (at which 97.8% of the short-component shuttings are too short to be detected), the values in table 1 suggest that τ and a'_i are affected. However, inspection of figure 6*c* shows that even with a 200 μs resolution, the shut-time distribution is not much distorted. In this case the figures in table 1 are misleading, because the asymptotic approximation becomes poor for short times, as shown in figure 7.

The result in figure 7*a* shows that the asymptotic open-time distribution fits very

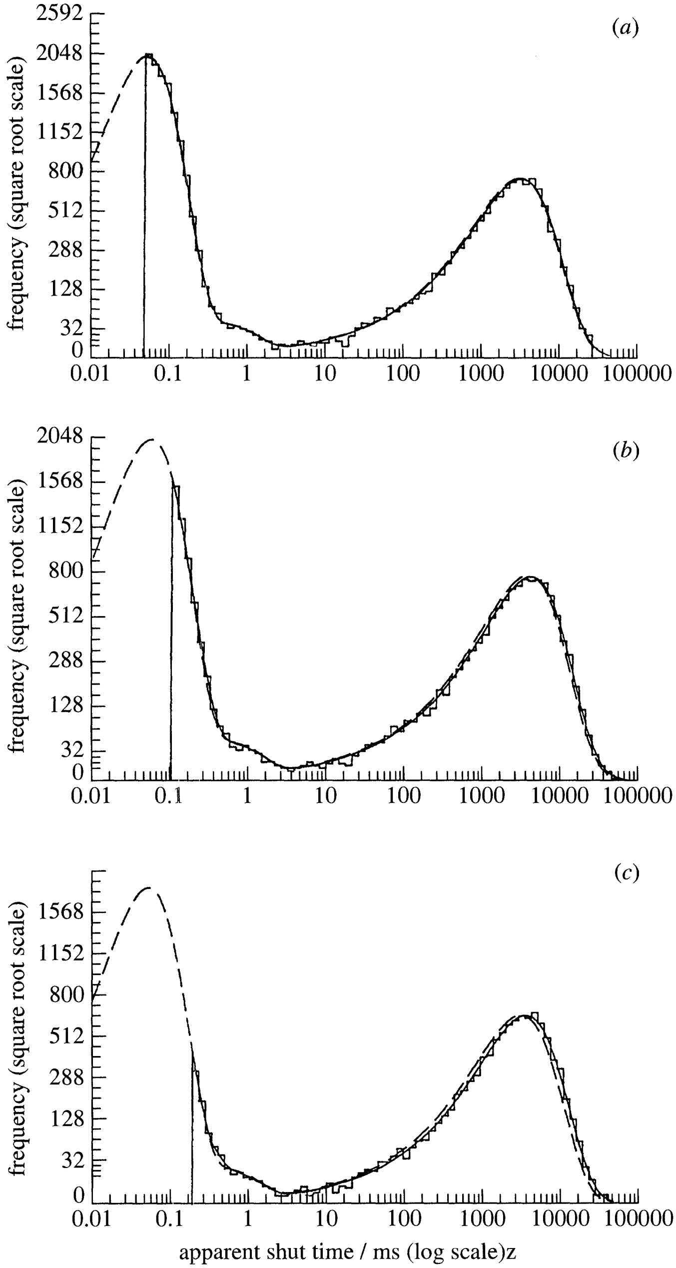


Figure 6. Distribution of apparent shut times, with resolutions of (*a*) 50 μs, (*b*) 100 μs and (*c*) 200 μs. The solid line shows the theoretical distribution; the exact calculation was used up to $t = 3\xi$, and the asymptotic form was used for larger t. The time constants and areas for the asymptotic form are given in table 1. The histogram shows simulated data, on which the appropriate resolution has been imposed (see text). The dashed line shows the ideal distribution (scaled to have the same area above $t = \xi$) in which no events are missed.

well right down to $t = \xi$; there is hardly any need for the exact solution in this case. However, the curves in figure 7*b* show that, when the resolution is very poor ($\xi = 200$ μs), the asymptotic approximation for shut times becomes inaccurate below

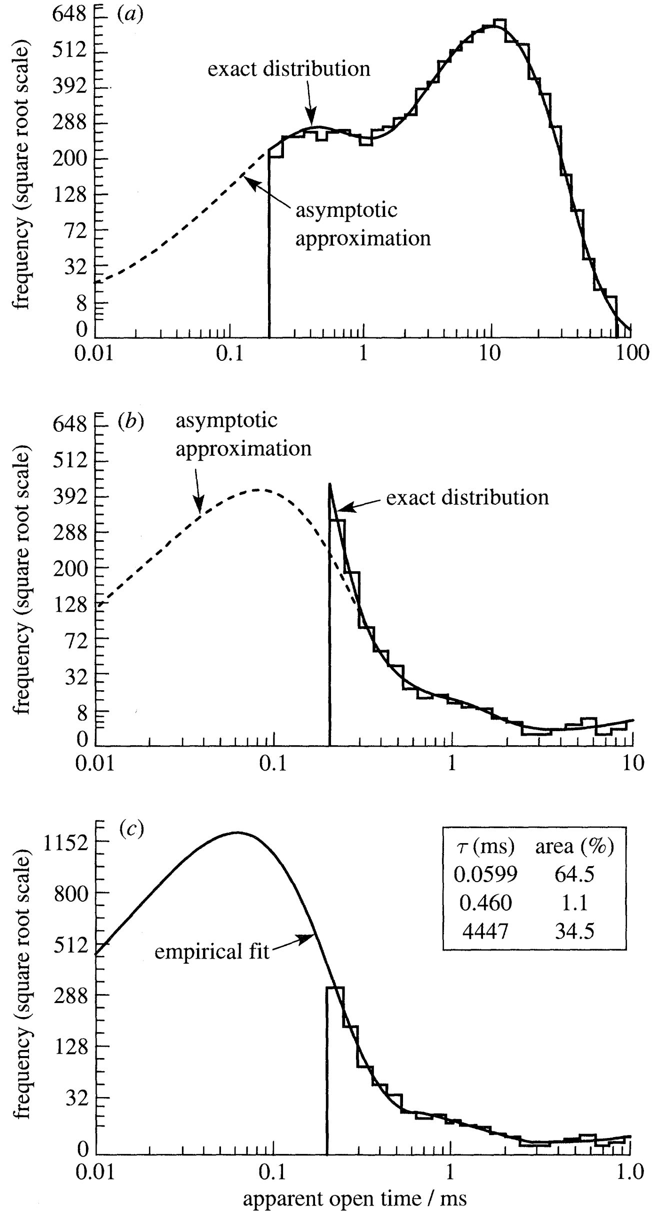


Figure 7. (a) Distribution of apparent open time with poor resolution, $\xi = 200\ \mu\text{s}$. The solid line shows the exact distribution from $t = 0.2$ ms to $t = 0.6$ ms, and the asymptotic form thereafter, as in figure 5c. The histogram is as in figure 5c. The dashed line shows the asymptotic form, projected back to $t = 0$. In this case the asymptotic form describes the data well over the whole range. (b) Distribution of apparent shut time with poor resolution, $\xi = 200\ \mu\text{s}$. The solid line and histogram are as in figure 6c, except that the distribution is shown only up to $t = 10$ ms, for clarity. The dashed line shows the asymptotic form, projected back to $t = 0$. In this case the asymptotic form is a good approximation only for $t > 0.3$ ms. (c). Empirical fit of a mixture of three exponentials to simulated shut data with a resolution of 200 μs, as shown in figure 6c. The fit was done by the conventional maximum likelihood method (see Colquhoun & Sigworth 1995). All values were included in the fit, but the result is shown here only up to 10 ms, to facilitate comparison with (b). The values for the fitted parameters are shown in the box.

$t = 2\xi$, though the exact distribution still fits the data well (and it is close to the ideal distribution, as shown in figure 6*c*). In figure 7, the asymptotic distributions are projected back to $t = 0$, though of course their derivation is based only on large t values, so the poor fit below $t = 2\xi$ is not entirely surprising. Below 600 μs (3ξ) the exact distribution has been used to calculate the shut time distribution, and this exact distribution is not a simple multiexponential. Nevertheless, it is interesting to see what happens when the simulated shut times with $\xi = 200$ μs, as shown in figure 6*c* and 7*b*, are fitted over the whole range with a mixture of three exponentials, because this is what would usually be done with real data. The result of doing this is shown, for the shorter shut times, in figure 7*c*. Not surprisingly, a good fit is obtained with three exponentials, and the fastest shut time component has a time constant of 59.9 μs. Even in this extreme case, the fitted time constants do not differ grossly from the ideal case ($\tau = 52.6$ μs for the fastest component).

The results in figures 5–7 show why the simple correction for missed events that was employed by Colquhoun & Sakmann (1985) worked quite well. The fact that the shut time distribution (unlike the open-time distribution) is relatively little distorted by missed events allows a fit like that shown in figure 7*c* to be projected back to $t = 0$ to obtain a reasonably accurate estimate of the number and duration of brief shuttings that have been missed. With this estimate, and the assumption that most missed shuttings occur adjacent to long openings (as suggested by the data), a reasonably accurate retrospective correction can be made.

Open time distribution conditional on adjacent shut-time range

In order to compare a theoretical distribution with experimental values, it is necessary to look at the distribution of open times that are adjacent to shut times in a specified *range* of values (equations (4.1) and (4.19)), rather than those adjacent to an exactly specified shut time (equations (3.2) and (3.17)). The graphs in figure 8 show examples of such conditional open-time distributions. The calculated distribution provides a good fit to the histogram of simulated observations in each case. In figures 8*a*, *c* the distribution of apparent open times that are adjacent to short shuttings are shown (for resolutions of 50 and 200 μs, respectively). In each case there is a deficiency of short openings, and an excess of long openings, compared with the unconditional distribution (which is shown as a dashed line in figure 8; it is what is plotted as a solid line in figure 5). Figures 8*b*, *d* show the distributions of apparent open times (for resolutions of 50 and 200 μs, respectively) that are adjacent to long shut times (any shut time longer than 10 ms). The results with $\xi = 50$ μs (figure 8*b*) show an excess of short openings, and a deficiency of long openings next to long shut times, but at the very poor resolution of 200 μs (figure 8*d*), this effect is barely visible.

As mentioned above, the time constants for the (asymptotic) conditional distributions of open time are exactly the same as those for the unconditional distribution. For example, with a resolution of 50 μs these are 0.3281 ms and 3.887 ms, and the (modified) areas for each of these are 0.131 and 0.869 (see table 1). The changed appearance of the conditional distribution is entirely a result of the dependence of the entry probabilities for each open state on the length of the adjacent shut times, and the consequent change in the relative (asymptotic) areas associated with each time constant. In the case where the resolution is 50 μs, the equilibrium probability that an apparent opening starts in open state 1 (AR^*) is 0.1187, and for state 2 (A_2R^*) it is 0.8813; these are the elements of $\phi_{\mathcal{A}}$ which were defined in (2.10). For

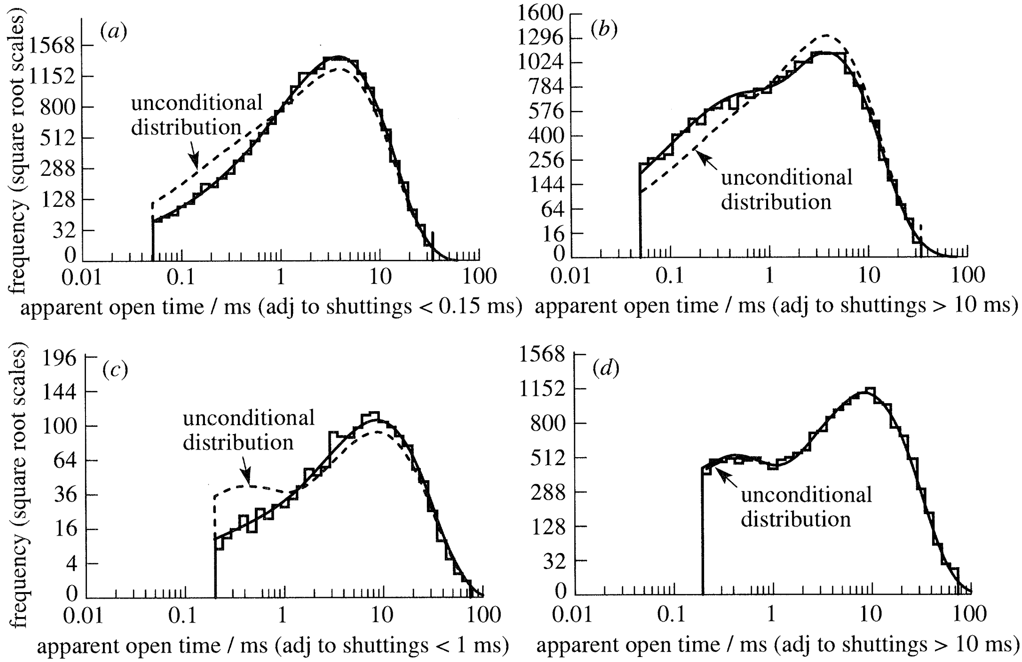


Figure 8. Distributions of apparent open times conditional on the length of the adjacent shut time. The histograms show the apparent open times that were preceded or followed by shut times in the specified range. The continuous lines show the distribution calculated from (4.1) for the distribution of open times conditional on the preceding shut time being in the specified range (this is indistinguishable from the corresponding distribution that is conditional on the *following* shut time—see comments preceding equation (3.8)). The dashed lines show the unconditional distributions of open time, as illustrated in figure 5. The resolution is 50 μs in (a) and (b), and 200 μs in (c) and (d). The distributions conditional on being adjacent to short shut times are shown in (a) and (c): in (a) the shut-time range is from 0.05 ms (the resolution) to 0.15 ms, and in (c) the shut-time range is 0.2 ms to 1 ms. The distributions conditional on being adjacent to long shut times are shown in (b) and (d): in both (b) and (d) all shut times longer than 10 ms are included in the range.

the distribution of open times conditional on a preceding shut-time range, the entry probabilities are given by the result following (4.7). For apparent open times preceded by apparent shuttings in the range 0.05–0.15 ms (figure 8a) the entry probabilities are 0.001 08 and 0.9989 for states 1 and 2, respectively, and the areas for each of the asymptotic time constants are 0.000 94 and 0.999 06. The reasons for these changes are easily explained. The brief shut times consist largely of sojourns in state 3 (A_2R) which is connected directly to open state 2 (A_2R^*) by a fast step, but state 3 is connected only indirectly (via slower steps) to open state 1. Thus, after a short interval, the next opening is much more likely (probability 0.9989) to start in state 2 than in state 1 (probability 0.001 08). Since sojourns in open state 2 are longer than those in open state 1, this results in the area of the long component of the conditional open-time distribution ($\tau = 3.887$ ms) being much larger (0.999 06) than the area for the faster time constant ($\tau = 0.3281$ ms) which has almost disappeared in figure 8a (area $a' = 0.000\,94$).

Conversely, for apparent openings that are preceded by long (greater than 10 ms) apparent shut times (figure 8b), the entry probabilities are 0.2468 and 0.7532 for states 1 and 2 (compared with 0.1187 and 0.8813 for the unconditional distribution of

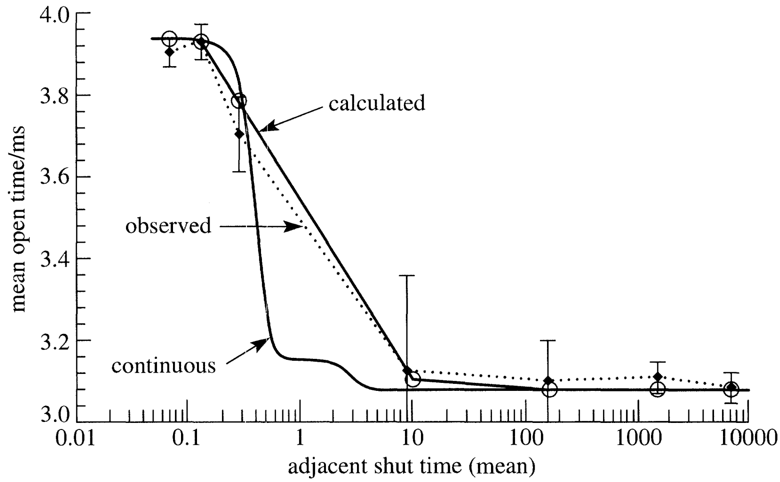


Figure 9. Dependence of the mean open time on the adjacent shut time, for a resolution of $\xi = 50\ \mu s$. The theoretical relationship, from equations (3.4) and (3.19), is shown as the curve labelled 'continuous' (the curves for preceding shut time and following shut time superimpose). The seven shut-time ranges that were used for comparison of the results with simulated data, together with the number (n) of apparent openings that were adjacent to shuttings in each range, were as follows: 0.05–0.1 ms ($n = 13\,928$); 0.1–0.2 ms ($n = 8106$); 0.2–1 ms ($n = 1766$); 1–30 ms ($n = 198$), 30–300 ms ($n = 1352$), 300–3000 ms ($n = 9360$), and longer than 3000 ms ($n = 9786$). The means of the apparent open times are plotted as filled diamonds (joined by dotted lines), with error bars that indicate the standard deviation of the mean (in each range the standard deviation of the open times is similar to its mean, so the differences in the size of the error bars are mainly a reflection of the number of shut times in each range). The mean open times are plotted against the mean of all shut times in the corresponding range. The calculated values (from equations (4.8) and (4.21), which are superimposed) are plotted as hollow circles (joined by straight lines).

apparent open times), and the asymptotic areas for the fast and slow components are 0.269 and 0.731 (compared with 0.131 and 0.869 for the unconditional distribution).

These conditional distributions show clearly the negative correlation between the length of an opening and the length of an adjacent shut time (this holds equally for the preceding shut time and the following shut time). This correlation can also be seen, in a different form, in the graphs in figures 9 and 10.

Dependence of mean open time on adjacent shut time

The correlation that was illustrated in figure 8, is also apparent when we plot the mean length of an opening against the length of an adjacent shut time. The theoretical relationship, from equations (3.4) and (3.19), is shown as the curve labelled 'continuous' in figure 9 (the curves for preceding shut time and following shut time superimpose). It has quite a subtle shape that should be useful for discrimination between possible mechanisms. However, the extent to which it can be compared with experimental observations is limited because, as is clear from figure 6, there are few shut times between 0.5 and 100 ms in length in this particular case. Again, for comparison with observations, it is necessary to use a range of adjacent shut times, and the mean value of open times that are adjacent to shut times in a specified range can be found from equations (4.8) and (4.21). These calculated values are shown, for seven different shut-time ranges, as hollow circles (joined by straight lines, labelled 'calculated') in figure 9. They are plotted against the mean of the shut times in the

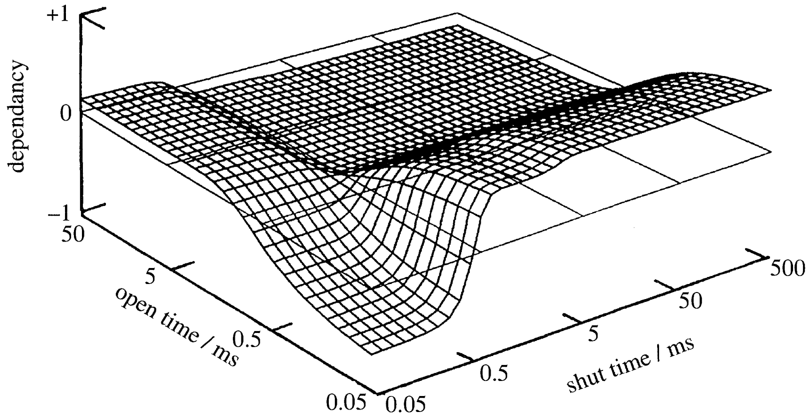


Figure 10. The calculated dependency plot with allowance for missed events, as defined in equation (3.22) is shown, for the mechanism in (6.1)–(6.3). The calculations were for a resolution of 50 μs. The axes extend from 50 μs to 50 ms for the apparent open time, and from 50 μs to 500 ms for the apparent shut time. The deficiency of short openings adjacent to short shut times is shown by the depression at the front corner of the graph, while the elevation at the right-hand corner shows the excess of short openings adjacent to long shut times.

corresponding shut-time range. The corresponding values for the observations are shown as filled diamonds (joined by dotted straight lines) in figure 9. The agreement between the simulated observations and the calculated values is good, given the relatively small number of shut times in some of the ranges.

Dependency plot

The dependency plot with allowance for missed events (as defined in equation (3.22)) is shown in figure 10, for the mechanism in (6.1)–(6.3). It is plotted for results with a resolution of 50 μs. It shows the deficiency of long openings adjacent to short shuttings (and conversely) in an attractive three-dimensional manner. However, it is necessary to have a large number of observations to produce the equivalent diagram from experimental results, especially in a case like the present for which some shut-time durations occur only rarely in the data. When the dependency plot is drawn for observations with lower resolution, the main effect (in this example at least) is to cut off the part of the graph for short times without having a dramatic effect on the rest of the graph.

Some examples of direct maximum likelihood fitting

The direct maximum likelihood method described in § 5 has been tested using simulated observations. For this purpose a record was generated with 5120 open times, 5120 shut times and a resolution of 50 μs. The likelihood is defined in (5.5), or, in the case where more than one channel is present, by (5.7)–(5.12). The likelihood was maximized by a Simplex method. The time taken for convergence depends enormously on the number of parameters, the number of observations and on the quality of the initial guesses; on a fast PC it may take a few minutes or up to an hour or more. In each case the quality of the fit would be judged, after the fitting was completed, by using the estimated parameters ($\boldsymbol{Q}$ matrix) to construct plots of the sort exemplified above.

Table 2. *Some examples of fitting for simulated data consisting of* 5120 *open times and* 5120 *shut times, with a resolution of* 50 μs, *in the case where it is assumed that only one channel contributes to the data*

(Constrained values are denoted with †. In each case the parameter $2k^*_{-2}$ is determined from the others by the microscopic reversibility (MR) constraint, so it is not estimated separately. The log(likelihood) values for the initial guesses, and the final maximised values are given at the bottom for each fit.)

			fit 1		fit 2		fit 3	
parameter	units	true value	initial guess	final estimate	initial guess	final estimate	initial guess	final estimate
α_1	s^{-1}	3000	10 000	2900	500	2747	1000	2845
β_1	s^{-1}	15	1000	16.9	3	15.8	3	6.685
k^*_{+2}	$M^{-1}\,s^{-1}$	5×10^8	5×10^7	$5.98 \times 10^8_{\dagger}$	15×10^8	$5.25 \times 10^8_{\dagger}$	5×10^7	1.87×10^8
$2k^*_{-2}$	s^{-1}	0.666 667	MR	0.786	MR	0.748	MR	0.2842
α_2	s^{-1}	500	100	514	100	500.4	5000	17 046
β_2	s^{-1}	15 000	30 000	15 789	3000	15 492	50 000	90 134
$2k_{-2}$	s^{-1}	4000	1000	4148	10 000	4010	1000	639.6
k_{+2}	$M^{-1}\,s^{-1}$	5×10^8	5×10^7	5.98×10^8	15×10^8	5.25×10^8	5×10^7	1.87×10^8
k_{-1}	s^{-1}	2000	1000	2015	10 000	2310	1000	1440
$2k_{+1}$	$M^{-1}\,s^{-1}$	1×10^8	1×10^7	0.86×10^8	5×10^8	1.08×10^8	1×10^7	1.54×10^8
log(likelihood)			22 423.5	38 790.2	33 640.4	38 791.1	30 995.4	38 677.4

Fitting with different initial guesses

Table 2 shows some examples of fits in cases where the openings were assumed to originate from one channel only, using different initial guesses for the parameters. Attempts to fit all the parameters (other than that determined by microscopic reversibility) usually led to values of k^*_{+2} and k^*_{-2} that were much too small (though in about the right ratio). Transitions between A_2R and A_2R^* are probably too rare for there to be much information about their frequencies in the data. However, if we constrain k^*_{+2} to be equal to k_{+2} then fitting is successful, though not from all initial guesses. The fits labelled 'fit 1' and 'fit 2' in table 2 started from very different initial guesses, but both give quite reasonable estimates of the rate constants. If, on the other hand, the initial guesses for both α_2 and β_2 were much too big then both of these parameters became even bigger, as illustrated in fit 3 (table 2), and the estimates of the other parameters were poor. This erroneous fit has attempted to fit long openings as though they consisted of a series of many short openings and even shorter shuttings. However, this sort of error is unlikely, because the apparent long open time (table 1 and figure 5*a*) is about 4 ms, the reciprocal of which, 250 s^{-1}, might make a sensible initial guess for α_2; so a guess of 5000 s^{-1}, as used in fit 3, would be rather implausible. In the simplest case of a mechanism with two states only, it has been shown that there are two different solutions to the missed events problem, one with much briefer open and shut times than the other: the behaviour of more complex models in this respect is not known, but it is possible that the poor fit seen in fit 3 reflects behaviour analogous with the 'fast solution' in the two-state case (see Colquhoun & Hawkes 1995*a*, p. 455).

Table 3. *Some examples of fitting for simulated data consisting of* 5120 *open times and* 5120 *shut times, with a resolution of* 50 μs, *in the case where the number of channels is not known*

(In all cases t_{crit} was taken as 5 ms. Constrained values are denoted with †, fixed values are denoted with ‡. In each case the parameter $2k^*_{-2}$ is determined from the others by the microscopic reversibility (MR) constraint, so it is not estimated separately. In the last column k_{-2} was constrained to be equal to k_{-1}, so only the latter was estimated.)

parameter	units	true value	initial guess	fit 1 final estimate	fit 2 final estimate	fit 3 final estimate
α_1	s^{-1}	3000	9000	3097	3337	2830
β_1	s^{-1}	15	5	34.7	10.2	14.7
k^*_{+2}	$M^{-1}\,s^{-1}$	5×10^8	5×10^8	9.62×10^7	$5 \times 10^8_{\ddagger}$	$5 \times 10^8_{\ddagger}$
$2k^*_{-2}$	s^{-1}	0.666 667	MR	0.105	0.413	0.681
α_2	s^{-1}	500	100	495.3	539.3	504.6
β_2	s^{-1}	15 000	5000	15 371	16 359	15 555
$2k_{-2}$	s^{-1}	4000	10 000	4356	4103	4036
k_{+2}	$M^{-1}\,s^{-1}$	5×10^8	1×10^9	1.4×10^9	$5 \times 10^8_{\ddagger}$	$5 \times 10^8_{\ddagger}$
k_{-1}	s^{-1}	2000	5000	2747	1607	$2018_{\dagger}$
$2k_{+1}$	$M^{-1}\,s^{-1}$	1×10^8	2×10^8	2.36×10^6	$1 \times 10^8_{\ddagger}$	$1 \times 10^8_{\ddagger}$

Fitting groups of openings when there is more than one channel

If the simulated observations were from a patch that contained an unknown number of channels, we could still be sure that openings separated by less than about 5 ms were almost certainly from the same channel: roughly speaking, the 52.6 μs component of shut times (see table 1), and the 0.485 ms component, are 'shut times within a single activation', whereas the 3789 ms component is 'between activations'. Table 3 gives some examples of fits obtained by taking $t_{\text{crit}} = 5$ ms in (5.7)–(5.12).

The initial guess for each of the parameters is shown in column 4 of table 3. In each case the parameter $2k^*_{-2}$ is determined from the others by the microscopic reversibility constraint, so it is not estimated separately. The final estimates are shown in columns 5–7. In the first of these (fit 1) all the parameters (apart from $2k^*_{-2}$) were free to vary. The estimates are of modest quality, and $2k_{+1}$ is quite wrong: this is to be expected since the estimate of the association rate constant for the first binding step will be determined to a large extent by the frequency of channel activations, and this is not available when the number of channels is unknown. The second set of estimates (fit 2) was found by fixing all of the association rate constants at their correct values, and the estimates are seen to be better. For the the third set of estimates (fit 3), in addition the value of $2k_{-2}$ was constrained to be twice that of k_{-1}, so only the latter was estimated. In this case the estimates are quite good. It can be concluded that the information in a steady-state record at a single agonist concentration is insufficient to determine all of the rate constants in $\boldsymbol{Q}$ when the number of channels is unknown. However, if some rates (particularly the association rate constants) can be supplied in advance, good estimates of the others can be found.

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