Asymptotic Distributions of Apparent Open Times and Shut Times in a Single Channel Record Allowing for the Omission of Brief Events

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SUMMARY

The openings and shuttings of individual ion channel molecules can be described by a Markov process with discrete states in continuous time. The predicted distributions of the durations of open times, shut times, bursts of openings, etc. are all described, in principle, by mixtures of exponential densities. In practice it is usually found that some of the open times, and the shut times, are too short to be detected reliably. If a fixed dead-time τ is assumed then it is possible to define, as an approximation to what is actually observed, an 'extended opening' or e-opening which starts with an opening of duration at least τ followed by any number of openings and shuttings, all the shut times being shorter than τ; the e-opening ends when a shut time longer than τ occurs. A similar definition is used for e-shut times. The probability densities, $f(t)$, of these extended times have previously been obtained as expressions which become progressively more complicated, and numerically unstable to compute, as $t \to \infty$. In this paper we present, for the two-state model, an alternative representation as an infinite series of which a small number of terms gives a very accurate approximation of $f(t)$ for large t. For the general model we present an asymptotic representation as a mixture of exponentials which is accurate for all except quite small values of t. Some simple model-independent corrections for missed events are discussed in relationship to the exact solutions.

1. INTRODUCTION

Single channel recording has a substantially better time resolution than the methods that preceded it, so it is not surprising that it has revealed rapid events in channel function that were previously unsuspected (e.g. Colquhoun & Sakmann 1985). However, experimental records always seem to show phenomena that are just too rapid to be resolved easily, whatever efforts are made to increase the resolution. The filtering effect of the recording apparatus is such that the rise-time (10–90%) of the observed signal, in response to a square input, is at best 30–35 µs. This means that an opening of the ion channel that is shorter than 20–25 µs will not be detectable given the noise which is necessarily present to some extent in the recording. The resolution is very often worse than this, up to 500 µs or more, depending on the signal:noise ratio in the experimental record and on the method used for its analysis (see Colquhoun & Sigworth (1983) for details). Events (openings or shuttings) of the channel that have a duration much shorter than the resolution will not be detected. Long events will always be detected whereas events of intermediate duration will be detected sometimes and not others. This will cause a potentially serious distortion of the results. Several approximate methods have been described for coping with this 'missed event' problem, for

example by Roux & Sauvé (1985), Blatz & Magleby (1986), Ball & Sansom (1988), Yeo et al. (1988) and Crouzy & Sigworth (1990). An exact solution to the problem was found by Hawkes et al. (1990); in this paper we discuss some forms that are easier to compute, and which approximate the exact solution closely.

It is supposed in what follows that all events that are shorter than some fixed resolution or dead-time (denote τ) are not detected, whereas all events that are longer than τ 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 τ with the open times on each side of it to produce one long 'apparent opening' (Colquhoun & Sigworth, 1983). 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'. Further discussion of the problem of resolution is given in Hawkes et al. (1990).

2. NOTATION AND BASIC RESULTS

The principles and notation are those employed by Hawkes et al. (1990). 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

Phil. Trans. R. Soc. Lond. B (1992) 337, 383–404
Printed in Great Britain

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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 Q. The elements of Q have the dimensions of reciprocal time, and the diagonal elements, q_{ii}, are defined so that the rows sum to zero, so $-1/q_{ii}$ is the mean lifetime of a sojourn in state i.

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

$$Q = \begin{bmatrix} Q_{\mathscr{A}\mathscr{A}} & Q_{\mathscr{A}\mathscr{F}} \\ Q_{\mathscr{F}\mathscr{A}} & Q_{\mathscr{F}\mathscr{F}} \end{bmatrix}. \tag{1}$$

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

$$G(t) = \begin{bmatrix} 0 & \exp(Q_{\mathscr{A}\mathscr{A}}t)Q_{\mathscr{A}\mathscr{F}} \\ \exp(Q_{\mathscr{F}\mathscr{F}}t)Q_{\mathscr{F}\mathscr{A}} & 0 \end{bmatrix}. \tag{2}$$

Thus each event is, alternately, the beginning of an open period or the beginning of a closed period. The element $g_{ij}(t)$ of this matrix is the probability density of the time to the next entry into a new subset and the probability that the state entered is j, conditional on starting in state i. The Laplace transform of this matrix will be denoted by

$$G^*(s) = \begin{bmatrix} 0 & G^*_{\mathscr{A}\mathscr{F}}(s) \\ G^*_{\mathscr{F}\mathscr{A}}(s) & 0 \end{bmatrix}, \tag{3}$$

where

$$G^*_{\mathscr{A}\mathscr{F}}(s) = (sI - Q_{\mathscr{A}\mathscr{A}})^{-1}Q_{\mathscr{A}\mathscr{F}};$$
$$G^*_{\mathscr{F}\mathscr{A}}(s) = (sI - Q_{\mathscr{F}\mathscr{F}})^{-1}Q_{\mathscr{F}\mathscr{A}}. \tag{4}$$

From these transition densities the open- and closed-time distributions are readily found. For example, the equilibrium distribution of open times has probability density function

$$f(t) = \phi_0 \exp(Q_{\mathscr{A}\mathscr{A}}t)Q_{\mathscr{A}\mathscr{F}}u_{\mathscr{F}}$$
$$= \phi_0 \exp(Q_{\mathscr{A}\mathscr{A}}t)(-Q_{\mathscr{A}\mathscr{A}})u_{\mathscr{A}}, \tag{5}$$

where

$$\phi_0 = p_{\mathscr{F}}(\infty)Q_{\mathscr{F}\mathscr{A}}/p_{\mathscr{F}}(\infty)Q_{\mathscr{F}\mathscr{A}}u_{\mathscr{A}}. \tag{6}$$

In these results, which were given by Colquhoun & Hawkes (1982), $p_{\mathscr{F}}(\infty)$ is the $\mathscr{F}$ partition of the vector of equilibrium probabilities (i.e. the fraction of receptors in each state at equilibrium), and the initial vector, ϕ_0, gives the equilibrium probabilities of an opening starting in each of the open states; $u_{\mathscr{A}}$ and $u_{\mathscr{F}}$ are vectors of units. Similar results can be obtained for shut times. By using the spectral expansion of the matrices $\exp(Q_{\mathscr{A}\mathscr{A}}t)$ and $\exp(Q_{\mathscr{F}\mathscr{F}}t)$, these distributions may be represented as mixtures of exponentials, assuming the Q-matrix corresponds to a time-reversible process (see Colquhoun & Hawkes 1982, pp. 24–25; Kelly 1979). When, as is usual, the eigenvalues are

distinct the numbers of components in the mixtures are, respectively, the numbers of open and shut states $k_{\mathscr{A}}$ and $k_{\mathscr{F}}$. Fitting mixtures of exponentials to observed histograms has therefore been used to obtain lower bounds for the numbers of open and shut states.

These distributions may be considerably distorted by an inability to detect very small intervals. We suppose a constant critical gap or dead-time, τ, such that open or shut periods of duration less than this are missed. One could take different dead-times for open and closed times, but it is not necessary in practice and complicates the theory. We suppose, after Colquhoun & Sigworth (1983), that an observable open time begins with a sojourn in the $\mathscr{A}$ states of duration at least τ and ends at the beginning of the next sojourn in $\mathscr{F}$ of duration greater than τ. Thus, the observed open time may consist of r shut times, each of duration less that τ, and $r + 1$ open times, of which the first must exceed τ. Observed shut times may be defined similarly.

Following Ball & Sansom (1988), we will consider a semi-Markov process whose events occur at time τ 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 will call e-open and e-closed intervals, are identical to the durations of the observed, or 'apparent', open and closed intervals, because we have taken the same τ to detect both open and closed periods (though in practice it is possible, in rare cases, that a rapid succession of short openings and shuttings could give rise to a signal that could not be measured unambiguously). These definitions are illustrated in figure 1.

Intervals of this process will be alternatively e-open and e-closed, so the transition densities will be given by a matrix of the form

$$^eG(t) = \begin{bmatrix} 0 & ^eG_{\mathscr{A}\mathscr{F}}(t) \\ ^eG_{\mathscr{F}\mathscr{A}}(t) & 0 \end{bmatrix}, \tag{7}$$

with Laplace transform

$$^eG^*(s) = \begin{bmatrix} 0 & ^eG^*_{\mathscr{A}\mathscr{F}}(s) \\ ^eG^*_{\mathscr{F}\mathscr{A}}(s) & 0 \end{bmatrix}. \tag{8}$$

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

$$^eG = \begin{bmatrix} 0 & ^eG_{\mathscr{A}\mathscr{F}} \\ ^eG_{\mathscr{F}\mathscr{A}} & 0 \end{bmatrix}. \tag{9}$$

Here we simplify the notion when setting $s = 0$ in a Laplace transform by omitting the '*' and the argument: for example, in this case $^eG^*_{\mathscr{A}\mathscr{F}}(0)$ is written as $^eG_{\mathscr{A}\mathscr{F}}$.

By looking only at alternate events, and ignoring the interval durations, we have a Markov chain on the $\mathscr{A}$ states with transition matrix $^eG_{\mathscr{A}\mathscr{F}}{}^eG_{\mathscr{F}\mathscr{A}}$ and equilibrium probability vector, $\phi_{\mathscr{A}}$, satisfying

$$\phi_{\mathscr{A}} = \phi_{\mathscr{A}}{}^eG_{\mathscr{A}\mathscr{F}}{}^eG_{\mathscr{F}\mathscr{A}}, \qquad \phi_{\mathscr{A}}u_{\mathscr{A}} = 1. \tag{10}$$
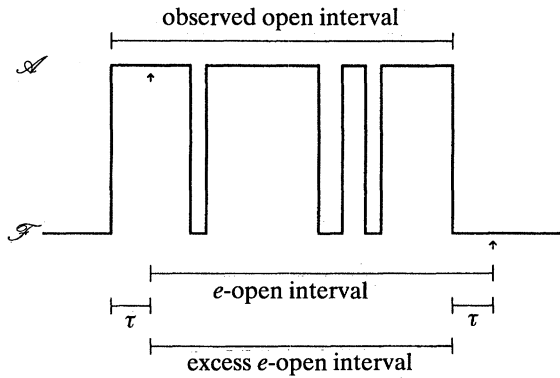
Figure 1. Illustration of the definition of an observed open interval, *e*-open interval and excess e-open interval for a channel with two levels of conductance and dead-time τ. The e-open interval is equivalent to an observed open interval shifted by an amount τ. The events of the semi-Markov process defined in the text occur at points marked ↑.

A Markov chain at the closed events has transition matrix ${}^{c}G_{\mathscr{F}\mathscr{A}}{}^{c}G_{\mathscr{A}\mathscr{F}}$ with equilibrium vector

$$\phi_{\mathscr{F}} = \phi_{\mathscr{A}}{}^{c}G_{\mathscr{A}\mathscr{F}}. \tag{11}$$

The solution of equation (10) for $\phi_{\mathscr{A}}$ can be found by rearranging in the form $\phi_{\mathscr{A}}(I - {}^{c}G_{\mathscr{A}\mathscr{F}}{}^{c}G_{\mathscr{F}\mathscr{A}}) = 0$, and then using the methods described by Colquhoun & Hawkes (1987) or Hawkes & Sykes (1990) for solution for equilibrium occupancies (the equation for which, $p(\infty)Q = 0$, has the same form). The results below, and those given by Hawkes *et al.* (1990), show that ${}^{c}G_{\mathscr{A}\mathscr{F}}$ can be evaluated as

$${}^{c}G_{\mathscr{A}\mathscr{F}} = \{I - G_{\mathscr{A}\mathscr{F}}(I - \exp(Q_{\mathscr{F}\mathscr{F}}\tau))G_{\mathscr{F}\mathscr{A}}\}^{-1} G_{\mathscr{A}\mathscr{F}}$$
$$\exp(Q_{\mathscr{F}\mathscr{F}}\tau),$$

and ${}^{c}G_{\mathscr{F}\mathscr{A}}$ is the same, except that $\mathscr{A}$ and $\mathscr{F}$ are interchanged.

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

$${}^{\mathscr{A}}R_{ij}(t) = P[X(t) = j \text{ and}$$

no shut time is detected over $(0,t)|X(0) = i]$, (12)

where a detectable shut time is a sojourn in $\mathscr{F}$ of duration greater than τ. 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 t and is currently in state j. Then the transition density is given by

$${}^{c}G_{\mathscr{A}\mathscr{F}}(t) = {}^{\mathscr{A}}R(t-\tau)Q_{\mathscr{A}\mathscr{F}}\exp(Q_{\mathscr{F}\mathscr{F}}\tau), \tag{13}$$

because, for the e-open interval to end at time t, there must be a transition from $\mathscr{A}$ to $\mathscr{F}$ at time $t-\tau$ (with no detectable sojourn in $\mathscr{F}$ up to that time) followed by a sojourn of at least τ in $\mathscr{F}$. ${}^{c}G_{\mathscr{A}\mathscr{F}}(t)$ is an important function as it enables one to write down a likelihood for an observed record (see Discussion), and

because the probability density of observed open times is given by

$$f_T(t) = \phi_{\mathscr{A}}{}^{c}G_{\mathscr{A}\mathscr{F}}(t)u_{\mathscr{F}}, \tag{14}$$

a result that resembles, in its notation, that found when events are not missed.

The distribution of e-open times covers the range $t = \tau$ to ∞ (see, for example, figure 3) because, by definition, any e-open time, T say, must exceed τ in duration. It is more convenient to consider the excess time $\tilde{T} = T - \tau$, which ranges from 0 to ∞. We will call this the excess e-open interval (see figure 1). Then the probability density function (PDF) $f_T(t) = f_{\tilde{T}}(t - \tau)$ and so

$$f_{\tilde{T}}(t) = \phi_{\mathscr{A}}{}^{c}G_{\mathscr{A}\mathscr{F}}(t + \tau) u_{\mathscr{F}}$$
$$= \phi_{\mathscr{A}}{}^{\mathscr{A}}R(t)Q_{\mathscr{A}\mathscr{F}}\exp(Q_{\mathscr{F}\mathscr{F}}\tau)u_{\mathscr{F}}. \tag{15}$$

It follows that ${}^{\mathscr{A}}R(t)$ is the key to the problem. Hawkes *et al.* (1990) show that its Laplace transform can be written as

$${}^{\mathscr{A}}R^{*}(s) = \{I - G_{\mathscr{A}\mathscr{F}}^{*}(s)S_{\mathscr{F}\mathscr{F}}^{*}(s)G_{\mathscr{F}\mathscr{A}}^{*}(s)\}^{-1}$$
$$(sI - Q_{\mathscr{A}\mathscr{A}})^{-1}, \tag{16}$$

where $S_{\mathscr{F}\mathscr{F}}^{*}(s)$ is defined by the equation

$$\int_{0}^{\tau} e^{-st} \exp(Q_{\mathscr{F}\mathscr{F}}t)\mathrm{d}t$$
$$= \{I - \exp(-(sI - Q_{\mathscr{F}\mathscr{F}})\tau)\}(sI - Q_{\mathscr{F}\mathscr{F}})^{-1}$$
$$= S_{\mathscr{F}\mathscr{F}}^{*}(s)(sI - Q_{\mathscr{F}\mathscr{F}})^{-1}. \tag{17}$$

Substituting equation (17) into equation (16), and using equation (4) yields the alternative expression

$${}^{\mathscr{A}}R^{*}(s) = \left[sI - Q_{\mathscr{A}\mathscr{A}} - Q_{\mathscr{A}\mathscr{F}}\left(\int_{0}^{\tau} e^{-st} \exp(Q_{\mathscr{F}\mathscr{F}}t)\mathrm{d}t \right) Q_{\mathscr{F}\mathscr{A}} \right]^{-1}. \tag{18}$$

These results were given, using different notation, by Ball & Sansom (1988), generalized to allow different critical intervals $\tau_{\mathscr{A}}$ and $\tau_{\mathscr{F}}$, which could be random variables, for open and closed intervals. They also gave general expressions for moments, which are easily obtained from equations (15) to (18).

(a) Probability density of excess e-open lifetimes

Hawkes *et al.* (1990) inverted the above transform, and hence obtained the pdf $f_{\tilde{T}}(t)$ in a form such that, for t in the interval $I_{n+1} = (n\tau, (n+1)\tau)$,

$$f_{\tilde{T}}(t) = \sum_{i=1}^{k} \theta_{in}(t)\exp(-\lambda_i t),$$

where $\theta_{in}(t)$ is a polynomial of degree n in t and $\lambda_1, \lambda_2, \ldots, \lambda_k$ are the eigenvalues of $-Q$. Thus, there is no single functional form, but a different form over each of the intervals I_n. Unfortunately the number of terms, and their complexity, increase as n increases. However, in this paper we find good approximations by simple forms for large t.

The general calculation of the above density is a little complicated, but the exact calculation is, as shown below, usually needed only for small t, for which the calculation is relatively simple. We therefore quote below the results for t in the ranges $t = 0$ to τ and $t = \tau$ to 2τ, which turn out to be adequate for practical purposes (these refer to excess times so they correspond to events with durations τ to 2τ, and 2τ to 3τ, respectively). The starting point is the representation of the matrix Q in terms of the spectral matrices A_i, see for example Colquhoun & Hawkes (1982), so

$$\exp(Qt) = \sum_{i=1}^{k} A_i \exp(-\lambda_i t). \tag{19}$$

Let $A_{i\mathscr{A}\mathscr{F}}$ be the $\mathscr{A}\mathscr{F}$ partition of A_i and define

$$D_i = A_{i\mathscr{A}\mathscr{F}} \exp(Q_{\mathscr{F}\mathscr{F}}\tau) Q_{\mathscr{F}\mathscr{A}}. \tag{20}$$

Then the required density is given by

$$\begin{aligned} f_{\bar{T}}(t) &= f_0(t) & 0 \leqslant t \leqslant \tau, \\ &= f_0(t) - f_1(t-\tau) & \tau \leqslant t \leqslant 2\tau, \end{aligned} \tag{21}$$

where

$$\left. \begin{aligned} f_0(t) &= \sum_{i=1}^{k} \gamma_{i00} \exp(-\lambda_i t), \\ f_1(t) &= \sum_{i=1}^{k} (\gamma_{i10} + \gamma_{i11} t) \exp(-\lambda_i t). \end{aligned} \right\} \tag{22}$$

The constants γ_{imr} are given by

$$\gamma_{imr} = \phi_{\mathscr{A}} C_{imr} Q_{\mathscr{A}\mathscr{F}} \exp(Q_{\mathscr{F}\mathscr{F}}\tau) u_{\mathscr{F}}, \tag{23}$$

where the matrices C_{imr} are given recursively by

$$\left. \begin{aligned} C_{i00} &= A_{i\mathscr{A}\mathscr{A}}, \\ C_{i10} &= \sum_{j \neq i} (D_i C_{j00} + D_j C_{i00})/(\lambda_j - \lambda_i), \\ C_{i11} &= D_i C_{i00}. \end{aligned} \right\} \tag{24}$$

(b) Individual openings

It may be of interest to know something about the individual openings that go to make up an e-opening. The theory is somewhat analogous to the study of bursts of openings in Colquhoun & Hawkes (1982). Let R be the number of openings in an e-opening, then its distribution is given by

$$\left. \begin{aligned} P(R = r) &= \phi_{\mathscr{A}} (G_{\mathscr{A}\mathscr{F}} S_{\mathscr{F}\mathscr{F}} G_{\mathscr{F}\mathscr{A}})^{r-1} \\ & \quad (I - G_{\mathscr{A}\mathscr{F}} S_{\mathscr{F}\mathscr{F}} G_{\mathscr{F}\mathscr{A}}) u_{\mathscr{A}}, \\ P(R \geqslant r) &= \phi_{\mathscr{A}} (G_{\mathscr{A}\mathscr{F}} S_{\mathscr{F}\mathscr{F}} G_{\mathscr{F}\mathscr{A}})^{r-1} u_{\mathscr{A}} \quad r \geqslant 1, \end{aligned} \right\} \tag{25}$$

the average number of openings being

$$E(R) = \phi_{\mathscr{A}} (I - G_{\mathscr{A}\mathscr{F}} S_{\mathscr{F}\mathscr{F}} G_{\mathscr{F}\mathscr{A}})^{-1} u_{\mathscr{A}}. \tag{26}$$

We note that, by definition, the first opening of an e-opening does not include the initial dead-time τ (see figure 1). Then the probability density of the lifetime, T_r, of the rth opening of an e-opening, given that it exists, is given by

$$\begin{aligned} f_r(t) &= -\phi_{\mathscr{A}} (G_{\mathscr{A}\mathscr{F}} S_{\mathscr{F}\mathscr{F}} G_{\mathscr{F}\mathscr{A}})^{r-1} \\ & \quad \exp(Q_{\mathscr{A}\mathscr{A}} t) Q_{\mathscr{A}\mathscr{A}} u_{\mathscr{A}}/P(R \geqslant r) \quad t > 0, \end{aligned} \tag{27}$$

whereas the entry probabilities at the start of the rth opening (i.e. the probabilities that it starts in each of the open states) are given by the elements of the vector

$$\phi_{\mathscr{A}} (G_{\mathscr{A}\mathscr{F}} S_{\mathscr{F}\mathscr{F}} G_{\mathscr{F}\mathscr{A}})^{r-1}/P(R \geqslant r). \tag{28}$$

The mean open times, starting from each possible open state, are given by the elements of the vector

$$-Q_{\mathscr{A}\mathscr{A}}^{-1} u_{\mathscr{A}}, \tag{29}$$

which, when averaged with respect to the probabilities in equation (28), gives the mean value of the distribution in equation (27) as

$$\begin{aligned} E(T_r) = \\ -\phi_{\mathscr{A}} (G_{\mathscr{A}\mathscr{F}} S_{\mathscr{F}\mathscr{F}} G_{\mathscr{F}\mathscr{A}})^{r-1} Q_{\mathscr{A}\mathscr{A}}^{-1} u_{\mathscr{A}}/P(R \geqslant r). \end{aligned} \tag{30}$$

An e-opening may contain some short shut times. The average total open time per e-opening, i.e. excluding the shut portion, is

$$\begin{aligned} E(T_{\text{open}}) &= \sum_{r=1}^{\infty} E(T_r) P(R \geqslant r) \\ &= -\phi_{\mathscr{A}} (I - G_{\mathscr{A}\mathscr{F}} S_{\mathscr{F}\mathscr{F}} G_{\mathscr{F}\mathscr{A}})^{-1} Q_{\mathscr{A}\mathscr{A}}^{-1} u_{\mathscr{A}}. \end{aligned} \tag{31}$$

In these equations, using our notational convention,

$$G_{\mathscr{A}\mathscr{F}} = G_{\mathscr{A}\mathscr{F}}^*(0) = -Q_{\mathscr{A}\mathscr{A}}^{-1} Q_{\mathscr{A}\mathscr{F}},$$

and

$$G_{\mathscr{F}\mathscr{A}} = G_{\mathscr{F}\mathscr{A}}^*(0) = -Q_{\mathscr{F}\mathscr{F}}^{-1} Q_{\mathscr{F}\mathscr{A}};$$

$$S_{\mathscr{F}\mathscr{F}} = S_{\mathscr{F}\mathscr{F}}^*(0) = (I - \exp(Q_{\mathscr{F}\mathscr{F}}\tau)).$$

Equivalent results for shut times are obtained by interchanging $\mathscr{A}$ and $\mathscr{F}$; in particular, $E(T_{\text{shut}})$ denotes the mean total shut time per e-shut time.

3. THE TWO-STATE MODEL

The simplest possible model, comprising just one open state and one closed state, has naturally received most attention in the literature. The Q-matrix can be written as

$$Q = \begin{bmatrix} -\alpha & \alpha \\ \beta & -\beta \end{bmatrix}$$

so $-Q$ has eigenvalues $\lambda_1 = 0$, $\lambda_2 = \alpha + \beta$. Both open and shut times consist of sojourns in single states so that, for example, the individual open times have negative exponential distributions with means $E(T_r) = 1/\alpha$, $\forall r \geqslant 1$. The mean of the first interval of the e-opening is the same as that of all the other openings, so that $E(T_{\text{open}}) = E(R)/\alpha$, despite being a continuation of the initial opening (duration τ) of the observed open interval: this is a well known property of the exponential distribution which was noted in the context of channel openings by Neher & Steinbach (1978). The distribution of the number of openings in an e-opening, from equation (25), is a simple geometric distribution.

In this case $\phi_{\mathscr{A}}$ and $u_{\mathscr{F}}$ are unit scalars, so equation (15) implies that

$$f_{\bar{T}}(t) = {}^eG_{\mathscr{A}\mathscr{F}}(t+\tau) = {}^{\mathscr{A}}R(t)\alpha e^{-\beta\tau}, \tag{32}$$

whereas equation (18) implies that the Laplace transform of $^{\mathscr{A}}R(t)$ is

$$^{\mathscr{A}}R^*(s) = \left[s + \alpha - \alpha\beta \int_0^\tau e^{-(s+\beta)t}\, dt \right]^{-1} = 1/W(s),\quad (33)$$

or, alternatively,

$$^{\mathscr{A}}R^*(s) = (s+\beta)/[s(s+\alpha+\beta) + \alpha\beta e^{-(s+\beta)\tau}],\quad (34)$$

where equation (33) defines the function $W(s)$.

Hawkes *et al.* (1990) obtained an exact expression for $^{\mathscr{A}}R(t)$, and hence $f_T(t)$, as a special case of that described in § 2 above, and compared it with various approximations which have appeared in the literature. They also indicated that the asymptotic behaviour of $^{\mathscr{A}}R(t)$, and hence of $f_T(t)$, for large t is governed by the roots of the denominator, $W(s)$, of $^{\mathscr{A}}R^*(s)$; for a general treatment of the relationship between asymptotic behaviour of a function and the poles of its Laplace Transform see Smith (1966). There is one real root $s_1 < 0$ which exceeds the real part of any other root. Thus it can be shown that as $t \to \infty$

$$f_T(t) \cong - as_1 e^{s_1 t},\quad (35)$$

where $0 < a < 1$. So we have a single exponential density with an area which is not unity. This is not a problem because this is the asymptotic behaviour of the pdf, $f_T(t)$: it is not an attempt to approximate the whole distribution. In practice we have found that it gives an extremely accurate approximation to the exact pdf for t larger than a few multiples of τ.

Jalali & Hawkes (1992a) have generalized this result to give a complete representation of $^{\mathscr{A}}R(t)$ by the following theorem.

(a) *Theorem*

In addition to one real root s_1, $W(s)$ has infinitely many complex conjugate pairs of roots $s_n = \sigma_n + i\omega_n$, $\bar{s}_n = \sigma_n - i\omega_n$ ($n \geqslant 2$), with $(\sigma_n = \mathrm{Re}\ s_n) < (s_1 = \sigma_1) < 0$. Then

$$^{\mathscr{A}}R^*(s) = 1/W(s) = \frac{w_1}{s - s_1} + \sum_{n=2}^\infty \left\{ \frac{w_n}{s - s_n} + \frac{\bar{w}_n}{s - \bar{s}_n} \right\},$$

where

$$w_n = (s_n + \beta)/[\alpha + \beta + 2s_n + \tau s_n(s_n + \alpha + \beta)].$$

The inversion of this simple form of $^{\mathscr{A}}R^*(s)$ leads to the representation of the probability density

$$f_T(t) = \sum_{n=1}^\infty (1/\mu_n)\exp(-t/\mu_n)[a_n\cos(\omega_n t) + b_n \sin(\omega_n t)],\quad (36)$$

where the time constants are given by

$$\mu_n = -1/\sigma_n,\quad (37)$$

and the areas are given by

$$a_1 = \alpha e^{-\beta\tau}\omega_1\mu_1,\ b_1 = 0,\quad (38)$$

corresponding to the real root, and for $n > 1$

$$a_n = 2\alpha e^{-\beta\tau}p_n\,\mu_n,$$

$$b_n = -2\alpha e^{-\beta\tau}q_n\,\mu_n,\quad \text{where}\quad \omega_n = p_n + iq_n.\quad (39)$$

To compute $f_T(t)$ we should expect to get a good approximation, at least for large t, by taking only a finite number of terms from the series given in equation (36), using those roots of $W(s)$ which have the largest real parts. The terms of the form $\exp(-t/\mu_n)$ die away fast if μ_n is very small, i.e. σ_n is very negative, while we also note that the coefficients a_n, b_n corresponding to large $|s_n|$ tend to zero.

(b) *Finding the roots*

The real root s_1 is easily found by Newton–Raphson iteration, treating $W(s)$ as a function of real s and starting the iteration at $s = 0$, or by bisection. The complex roots are more difficult. There are three stages.

(i) *The exploration stage*

To simplify the problem slightly, let

$$\gamma = \beta\tau,\qquad \delta = (\alpha/\beta) - 1,\qquad x = 1 + s/\beta.\quad (40)$$

Then equations (33) and (34) suggest the definition of $h(x)$ as

$$(s+\beta)W(s)/\beta^2 \equiv h(x) = x(x+\delta) - (1+\delta)(1 - e^{-\gamma x}).\quad (41)$$

The roots of $h(x)$ are equivalent to those of $W(s)$ but with an additional root at $x = 0$, corresponding to $s = -\beta$. Let $x = u + iv$, where we need consider only $v > 0$. If this is a root of $h(x)$ it satisfies $h(x) = 0$ or, taking the real and imaginary parts separately,

$$u(u+\delta) - v^2 - (1+\delta)(1 - e^{-\gamma u}\cos\gamma v) = 0,\quad (42)$$

$$v(2u+\delta) - (1+\delta)e^{-\gamma u}\sin\delta v = 0.\quad (43)$$

Eliminating the trigonometrical terms between these gives

$$[u(u+\delta) - v^2 - (1+\delta)]^2 + [v(2u+\delta)]^2 = (1+\delta)^2 e^{-2\gamma u},\quad (44)$$

while

$$\tan\gamma v = -v(2u+\delta)/[u(u+\delta) - v^2 - (1+\delta)].\quad (45)$$

Equation (44) can be written as a quadratic in v^2; one of the solutions for v^2 is negative and so clearly of no use and, as we only need to consider positive values of v, we define the solution as a function of u to be

$$v = \phi(u) = \{ -[(1+\delta/2)^2 + (u+\delta/2)^2] + [(1+\delta)^2 e^{-2\gamma u} + 4(1+\delta/2)^2(u+\delta/2)^2]^{\frac{1}{2}}\}^{\frac{1}{2}},\quad (46)$$

provided the result is real, i.e. if the expression in braces is non-negative. Similarly, equation (45) can be written as a quadratic in u whose two solutions are, by definition

$$u = \psi_1(v) = -[\delta/2 + v/\tan\gamma v] - [(1+\delta/2)^2 + v^2/\sin^2\gamma v]^{\frac{1}{2}},\quad (47)$$

$$u = \psi_2(v) = -[\delta/2 + v/\tan\gamma v] + [(1+\delta/2)^2 + v^2/\sin^2\gamma v]^{\frac{1}{2}}.\quad (48)$$

It can be shown that $u < 0$ so that $\psi_1(v)$ is always valid whereas $\psi_2(v)$ may or may not be valid, depending on the parameter values. Therefore, to find roots whose real parts are greater than some arbitrary value, u_T,

i.e. u lies in the range $u_T < u < 0$, we define truncated functions

$$\psi_i^T(v) = \max(u_T, \min(0, \psi_i(v))), \qquad i = 1, 2. \qquad (49)$$

Then we plot the function $\phi(u)$, $u_T < u < 0$, and the two functions $\psi_i^T(v)$, $0 < v < \max(\phi(u); u \in (u_T, 0))$, on the same graph. Any point (u,v) which is a point of intersection of $\phi(u)$ with either of the other two, except if $u = u_T$ or $u = 0$, is a potential solution.

(ii) The improvement stage

These graphically obtained potential solutions will, of course, only be approximate and must be improved. Two methods are available:

Newton–Raphson. Given an approximate solution (u_n, v_n) of equations (42) and (43), the improved solution is given by

$$\begin{pmatrix} u_{n+1} \\ v_{n+1} \end{pmatrix} = \begin{pmatrix} u_n \\ v_n \end{pmatrix} - D^{-1} \begin{pmatrix} u_n(u_n + \delta) - v_n^2 - (1 + \delta)(1 - e^{-\gamma u_n} \cos \gamma v_n) \\ v_n(2u_n + \delta) - (1 + \delta)e^{-\gamma u_n} \sin \gamma v_n \end{pmatrix} \qquad (50)$$

where

$$D = \begin{pmatrix} 2u_n + \delta - \gamma(1 + \delta)e^{-\gamma u_n} \cos \gamma v_n & -2v_n - \gamma(1 + \delta)e^{-\gamma u_n} \sin \gamma v_n \\ 2v_n + \gamma(1 + \delta)e^{-\gamma u_n} \sin \gamma v_n & 2u_n + \delta - \gamma(1 + \delta)e^{-\gamma u_n} \cos \gamma v_n \end{pmatrix}. \qquad (51)$$

Starting from a point given by the exploratory graphical method, this will sometimes converge quite quickly to an accurate solution. In case of any difficulty one may use instead a bisection method.

Bisection method. Having identified a potential solution based on the intersection of $\phi(u)$ and $\psi_i(v)$, say, with u lying in some small interval (u_L, u_M), one can easily find the root u of the equation $u - \psi_i(\phi(u)) = 0$ within this interval by the usual bisection method. Then v is given by $\phi(u)$.

(iii) The checking stage

As we have manipulated the equations in a non-linear way, it is as well to check by substitution into equations (42) and (43) that a potential solution is not spurious. We have found in practice that about half of the potential solutions found in this way are spurious and the rest are actual roots.

Having obtained the real root, s_1, and a finite number of complex roots, $s_n = \beta(x_n - 1)$ where $x_n = u_n + iv_n$, we use equation (36) to compute the density $f_T(t)$, using a finite series of terms instead of the infinite series. The accuracy, which is typically very good for all except fairly small values of t, can be checked against the exact method of calculating $f_T(t)$ discussed in § 2. Our recommendation is to use the latter, which is in principle exact, for $t < \tau$ at least and switch over to the above approximation after some range of t for which the two forms agree closely. This change-over point can be made smaller by finding more roots (by decreasing the cutoff value u_T used in the root-finding procedure).

4. NUMERICAL EXAMPLES WITH TWO STATES

(a) Examples

In this section we study three examples, starting with

two models from Colquhoun & Sigworth (1983). We will use milliseconds as the unit of time throughout.

Model CSF. A 'fast' model with dead-time $\tau = 0.2$ ms and (true) mean open and closed lifetimes given by $\mu_o = 1/\alpha = 0.1063$ ms, $\mu_c = 1/\beta = 0.2148$ ms.

Model CSS. A 'slow' model with the same dead-time τ as above but with (true) mean lifetimes $\mu_o = 0.2990$ ms, $\mu_c = 0.8787$ ms.

These two sets of values are of interest because, despite the fact that they are quite different, they both give identical values for the observed mean open and closed times (0.6 ms and 2.0 ms respectively) when events shorter than 0.2 ms are missed (the distributions, however, are not identical). It is worth noting, at this point, that the values of 0.6 ms and 2.0 ms are

the means of the distribution of e-open times and of e-shut times, respectively. They are, therefore, in a sense 'too long', because values less than τ are omitted (they are what one would get by simply averaging a large number of observed values). If data that obeyed either of these models were fitted, over the range where they were adequately described by a single exponential, then most fitting methods (e.g. the maximum likelihood method; Colquhoun & Sigworth 1983), would give results that corresponds to the mean excess open and shut times, namely $0.6 - \tau = 0.4$ ms, and $2.0 - \tau = 1.8$ ms (the results in tables 1–4 show that the single-exponential approximations to the true PDFs have time constants that are close to these values, especially for the slower CSS model). This happens because the fitting process effectively extrapolates the fitted curve to $t = 0$, and so includes (an estimate of) the e-openings that were too short to be seen. The result is close to the excess time because, for a single exponential, it follows from equation (69) that the mean of all values is less, by an amount τ, than the mean of only those values that exceed τ.

For the fast model the mean open time is less than the dead-time, whereas the mean closed time only just exceeds it. For the slow model both mean lifetimes are greater than the dead-time. However, an observed open or shut time will tend to consist of more intervals in the fast model ($E(R) = 2.537$ for openings and 6.563 for shuttings) than in the slow model ($E(R) = 1.256$ and 1.952). Consequently, $E(T_{open}) = 0.270$ ms, 0.375 ms respectively for the fast and slow models (compare with mean excess e-open time $0.6 - \tau = 0.4$ ms) whereas the equivalent results for shut times are $E(T_{shut}) = 1.410$ ms, 1.715 ms (compare with 1.8 ms): this is a relatively small fraction for the fast model. Note that, in the case of e-shut intervals in the fast model, $P(R \geqslant 42) > 0.001$ so that more than one e-shut

of finding the roots of $W(s)$ as described in the previous section (note that we find it convenient to plot v against $-u$). We see from equation (47) that the function ψ_1 has infinitely many branches with asymptotes at $v = n\pi/\gamma$ for all positive integers n. The potential roots are given by the intersections of this with the ϕ curve. It happened that the Newton–Raphson procedure failed on half the potential roots, and these were found using bisection: at the checking stage it turned out that these potential solutions did not satisfy the original equations, so this failure is hardly surprising. Note that it may be necessary to start with quite a narrow interval of u values when using the bisection method, to avoid jumping onto another branch of the ψ_1 function. The actual roots, marked on the graph with an asterisk, occurred on alternate branches of the ψ_1 function in every example we have studied, so that (reverting to the original units) there is one frequency ω within every cycle of $2\pi/\tau$. No roots have been found on the ψ_2 function in any of our examples.

By using the one real root and the first five complex conjugate pairs of roots, the coefficients needed for the first few terms of equation (36) are given in the top half of table 1. Figure 2*b* shows the exact probability density of e-open times, computed according to the method of Hawkes *et al.* (1990), see § 2, the asymptotic exponential arising from the real root and the asymptotic expression arising from equation (36) using the exponential and the five damped oscillations. Note that, of course, the density is zero for $t < \tau$ because all e-open times must, by definition, last for at least as long as the dead-time. The exponential appears quite good for $t > 2\tau = 0.4$, whereas the six-term approximation appears good down to about $t = 0.22$.

To see how the accuracy improves for small t as we add more terms, we show, in figure 2*c*, several approximations to the probability density, $f_{\tilde{T}}(t)$, of the excess time $\tilde{T} = T - \tau$ (using one, two, four and six roots: it is too messy to show them all) for t in the range $(0, \tau)$. Note that the approximations appear to be getting worse at the origin itself: as with Fourier series, we expect that the limiting value will be the average of the values of the density on the right and on the left of the origin (the latter being zero). One cannot see much from the graphs about the accuracy for large t, and so a summary is given in the lower half of table 1. Over each of several ranges of t, expressed as multiples of τ, we show the maximum absolute percentage error of each approximation relative to the exact density of $\tilde{T}$. As expected, the exponential approximation is poor for $t < \tau$ but the error is less than 0.053% for $t > 2\tau$. As we move to the right in the table, adding more terms to the series, we see the accuracy steadily improving and reaching levels of the order of $10^{-9}\%$.

A similar analysis is carried out for the distribution of e-shut times arising from model CSF, and reported in table 2 and figure 3 (figures corresponding to figures 2(*a,c*) tend to look quite similar for all examples so we do not include them routinely). We note that the same general pattern exists but, with shut times being longer on average, the distribution is
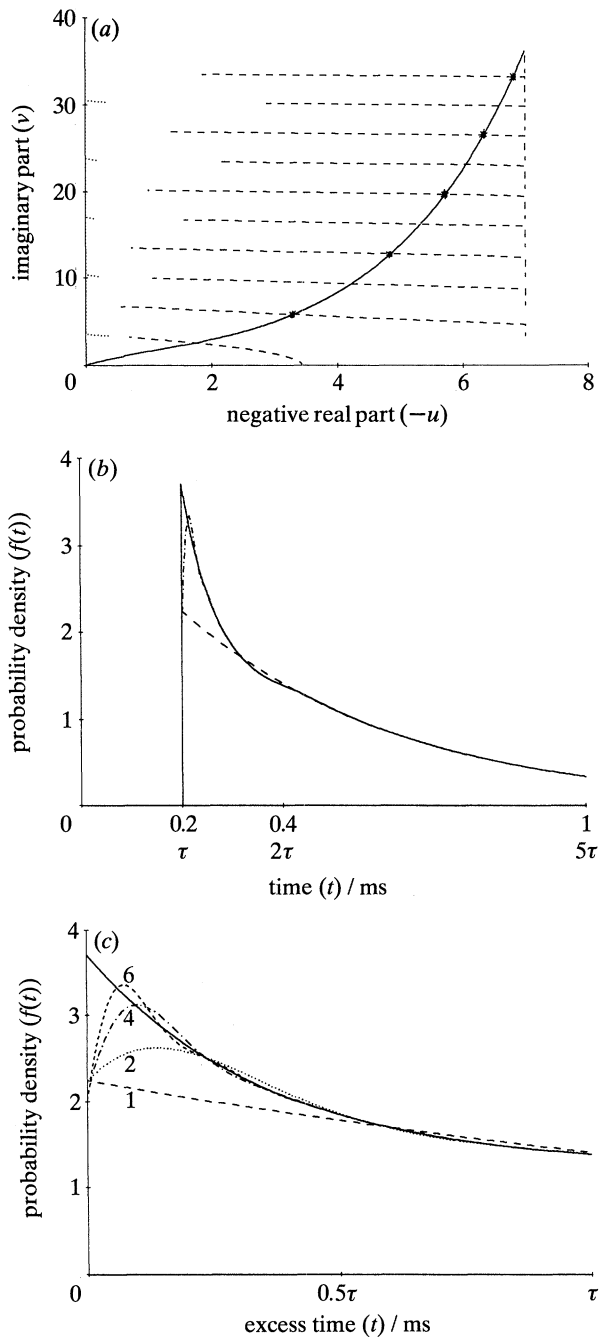
Figure 2. Distribution of e-open times in model CSF. (*a*) Preliminary root-finding plot. Potential roots are the intersections of $v = \phi(u)$ (solid line) and the function $u = \psi_1^{\mathrm{T}}(v)$ truncated at $u_{\mathrm{T}} = -7$ (dashed line); note that we find it convenient to plot negative values of u along the horizontal axis. Only half the intersections, marked by *, are actually roots. The function $\psi_2^{\mathrm{T}}(v)$, shown dotted on the left, does not have any intersections. (*b*) The exact probability density, $f_T(t)$, of the full observed open times, computed as indicated in Hawkes *et al.* (1990), as a solid line, together with the first (dashed) and sixth (dot-dashed) asymptotic approximations. (*c*) The exact density, $f_{\tilde{T}}(t)$, of the excess e-open times and the first, second, fourth and sixth asymptotic approximations over the range $0 < t < \tau$.

time in a thousand could contain at least 42 individual openings!

First we study the distribution of e-open times for the CSF model. Figure 2*a* shows the exploration stage

Table 1. *Model CSF: open times*

(The top half of the table gives the parameters of the first six components of the asymptotic density of excess observed lifetimes, $\tilde{T} = T - \tau$, as defined by equation (36). The lower half of the table shows the maximum absolute percentage error of the asymptotic forms relative to the exact density, over various ranges of t: each successive asymptote is obtained by adding one extra component to the previous one.)

component	1	2	3	4	5	6
μ/ms	0.42119	0.05006	0.03673	0.03195	0.02926	0.02748
ω/(rad. ms^{-1})	0	27.175	59.602	91.673	123.51	155.21
area						
a	0.94643	-0.00061	-0.00288	-0.00163	-0.00103	-0.00071
b	0	0.06691	0.02236	0.01268	0.00865	0.00648
t value range			maximum error (%)			
$0-\tau/4$	39	40	42	43	44	45
$\tau/4-\tau/2$	19	2.9	2.7	1.2	1.1	0.72
$\tau/2-3\tau/4$	3.4	0.80	0.37	0.21	0.14	0.099
$3\tau/4-\tau$	3.2	0.51	0.19	0.083	0.050	0.028
$\tau-2\tau$	1.5	0.19	0.059	0.026	0.018	0.012
$2\tau-3\tau$	0.053	1.5×10^{-3}	2.0×10^{-4}	5.0×10^{-5}	1.7×10^{-5}	6.8×10^{-6}
$3\tau-4\tau$	1.0×10^{-3}	8.6×10^{-6}	6.0×10^{-7}	8.7×10^{-8}	1.9×10^{-8}	5.5×10^{-9}

Table 2. *Model CSF: shut times*

(Description as for table 1.)

component	1	2	3	4	5	6
μ/ms	1.8133	0.03999	0.03112	0.02767	0.02565	0.02428
ω/(rad. ms^{-1})	0	25.513	58.842	91.172	123.13	154.91
area						
a	0.99314	-0.00311	-0.00097	-0.00047	-0.00028	-0.00018
b	0	0.00930	0.00341	0.00203	0.00142	0.00108
t value range			maximum error (%)			
$0-\tau/4$	23	34	38	41	42	43
$\tau/4-\tau/2$	9.8	1.3	1.7	0.66	0.71	0.42
$\tau/2-3\tau/4$	2.6	0.41	0.13	0.074	0.048	0.037
$3\tau/4-\tau$	0.56	0.17	0.047	0.025	0.013	8.5×10^{-3}
$\tau-2\tau$	0.38	0.048	0.015	6.4×10^{-3}	3.4×10^{-3}	2.3×10^{-3}
$2\tau-3\tau$	1.3×10^{-3}	7.7×10^{-5}	1.2×10^{-5}	3.1×10^{-6}	1.1×10^{-6}	4.4×10^{-7}
$3\tau-4\tau$	1.2×10^{-5}	8.3×10^{-8}	7.7×10^{-9}	1.3×10^{-9}	3.0×10^{-10}	8.9×10^{-11}
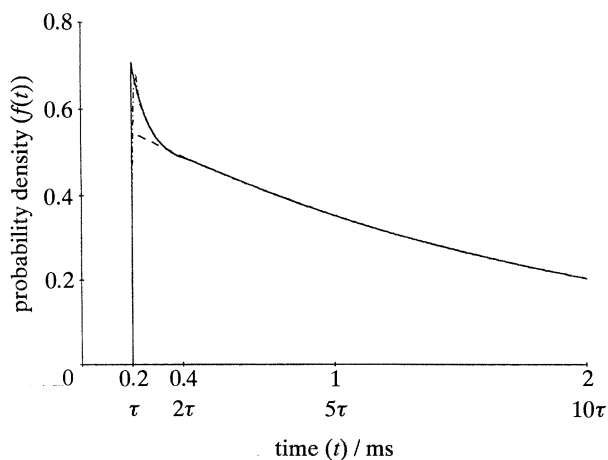
Figure 3. Probability density of full e-shut times, $f_T(t)$, in model CSF, together with the first (dashed) and sixth (dot-dashed) asymptotic approximations.

more nearly exponential: the area a corresponding to the first component being closer to 1 and the asymptotic approximations more accurate.

Turning now to the slow model, CSS, we expect the distributions of e-intervals to be broadly similar to those for the CSF model because they have the same means. There are, however, some differences as shown in Hawkes *et al.* (1990). The analyses are given in table 3 and figure 4 for e-open times (compare with figure 2b) and in table 4 and figure 5b for e-shut times (compare figure 3). We include also another example of the initial root finding plot in figure 5a), showing more potential roots, using truncation point $u_T = -15$, although we have used only five of them.

It is evident from the figures that the slower process is much more nearly exponential, and the area, a, for the exponential coefficient closer to 1 in tables 3 and 4 than in tables 1 and 2; the percentage errors of the

Table 3. *Model CSS: open times*

(Description as for table 1.)

component	1	2	3	4	5	6
μ/ms	0.40453	0.03304	0.02741	0.02481	0.02322	0.02211
ω/(rad. ms^{-1})	0	22.489	57.462	90.259	122.45	154.36
area						
a	0.98754	-0.01898	-0.00442	-0.00201	-0.00116	-0.00076
b	0	0.02485	0.01094	0.00673	0.00477	0.00366

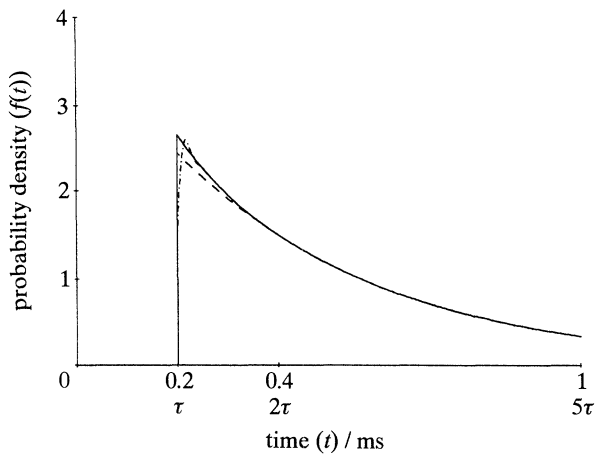| t value range	maximum error (%)					
$0-\tau/4$	8.4	30	36	39	41	42
$\tau/4-\tau/2$	4.7	0.76	1.3	0.49	0.58	0.32
$\tau/2-3\tau/4$	2.0	0.34	0.11	0.051	0.033	0.024
$3\tau/4-\tau$	0.35	0.11	0.027	0.016	8.1×10^{-3}	5.5×10^{-3}
$\tau-2\tau$	0.13	0.029	9.6×10^{-3}	4.2×10^{-3}	2.2×10^{-3}	1.3×10^{-3}
$2\tau-3\tau$	4.9×10^{-3}	1.7×10^{-5}	4.1×10^{-6}	1.2×10^{-6}	4.3×10^{-7}	1.8×10^{-7}
$3\tau-4\tau$	8.3×10^{-7}	9.8×10^{-9}	1.3×10^{-9}	2.6×10^{-10}	7.1×10^{-11}	2.3×10^{-11}

Figure 4. Probability density of full e-open times, $f_T(t)$, in model CSS, together with the first (dashed) and sixth (dot-dashed) asymptotic approximations.

various asymptotic approximations are also less. Except for component 2, the frequencies of the oscillations are very similar for the two models but they damp down slightly faster (smaller μ_n) in the slow model.

We consider one further example in which one process is much slower than the other. The example was discussed by Blatz & Magleby (1986).

Model BM2. Take dead-time $\tau = 0.1$ ms and $\alpha = 1$, $\beta = 10$ so that the mean open time, $\mu_o = 1/\alpha = 1$ ms, is much longer than τ while the mean shut time, $\mu_c = 1/\beta = 0.1$ ms, is equal to τ.

The observed open and shut times are increased, by missing short events, to 2.89 ms and 0.216 ms, respectively. Clearly, open intervals are less likely to be missed than shut ones ($E(R) = 1.105$ shut times per e-shut time) and so the mean excess observed shut time, $0.216 - 0.1 = 0.116$ ms, is not much more than

Table 4. *Model CSS: shut times*

(Description as for table 1.)

component	1	2	3	4	5	6
μ/ms	1.8030	0.03034	0.02575	0.02349	0.02207	0.02107
ω/(rad. ms^{-1})	0	21.922	57.140	90.038	122.28	154.23
area						
a	0.99828	-0.00399	-0.00102	-0.00047	-0.00027	-0.00018
b	0	0.00411	0.00213	0.00135	0.00098	0.00075

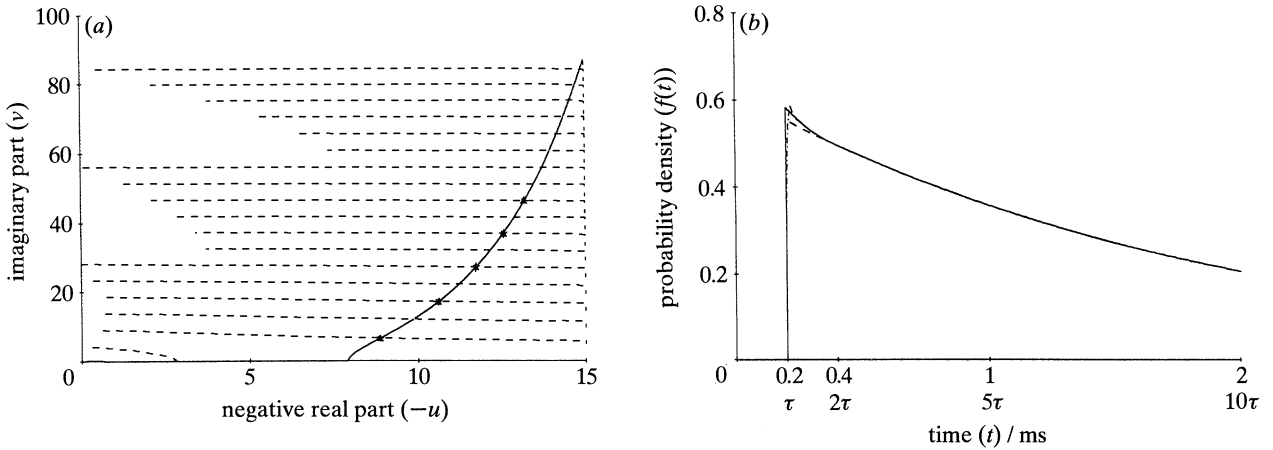| t value range	maximum error (%)					
$0-\tau/4$	5.0	28	34	38	40	41
$\tau/4-\tau/2$	2.7	0.56	1.0	0.37	0.46	0.25
$\tau/2-3\tau/4$	1.1	0.23	0.077	0.035	0.019	0.015
$3\tau/4-\tau$	0.21	0.054	0.013	8.8×10^{-3}	3.8×10^{-3}	2.9×10^{-3}
$\tau-2\tau$	0.036	0.012	4.1×10^{-3}	1.9×10^{-3}	9.9×10^{-4}	5.9×10^{-4}
$2\tau-3\tau$	7.7×10^{-5}	2.5×10^{-6}	7.2×10^{-7}	2.2×10^{-7}	8.1×10^{-8}	3.5×10^{-8}
$3\tau-4\tau$	2.1×10^{-8}	1.1×10^{-9}	8.6×10^{-11}	1.9×10^{-11}	5.5×10^{-12}	1.8×10^{-12}

Figure 5. Distribution of e-shut times in model CSS. In (a) the root finding plot shows more potential roots than in figure 2, using truncation point $u_T = -15$, and $\psi_2^T(v)$ is completely truncated. Only the first five complex roots have been used. In (b) is plotted the probability density of full e-shut times, $f_T(t)$, together with the first (dashed) and sixth (dot-dashed) asymptotic approximations.

Table 5. *Model BM2: open times*

(Description as for table 1.)

component	1	2	3	4	5	6
μ/ms	2.7909	0.01335	0.01168	0.01076	0.01018	0.00975
ω/(rad. ms^{-1})	0	42.427	113.37	179.44	244.07	308.06
area						
a	0.99972	-0.00112	-0.00032	-0.00015	-0.00009	-0.00006
b	0	0.00091	0.00057	0.00038	0.00028	0.00022

t value range	maximum error (%)					
$0-\tau/4$	2.6	25	33	37	39	41
$\tau/4-\tau/2$	1.3	0.54	0.80	0.27	0.37	0.19
$\tau/2-3\tau/4$	0.53	0.15	0.052	0.024	0.013	8.9×10^{-3}
$3\tau/4-\tau$	0.11	0.026	6.0×10^{-3}	4.6×10^{-3}	1.9×10^{-3}	1.6×10^{-3}
$\tau-2\tau$	8.6×10^{-3}	4.9×10^{-3}	1.8×10^{-3}	8.3×10^{-4}	4.5×10^{-4}	2.7×10^{-4}
$2\tau-3\tau$	9.5×10^{-6}	3.3×10^{-7}	1.2×10^{-7}	4.0×10^{-8}	1.5×10^{-8}	6.7×10^{-9}
$3\tau-4\tau$	3.7×10^{-9}	1.0×10^{-10}	5.6×10^{-12}	1.3×10^{-12}	4.0×10^{-13}	1.2×10^{-13}

Figure 6. Probability density of full e-open times, $f_T(t)$, in model BM2, together with the first (dashed) and sixth (dot-dashed) asymptotic approximations.

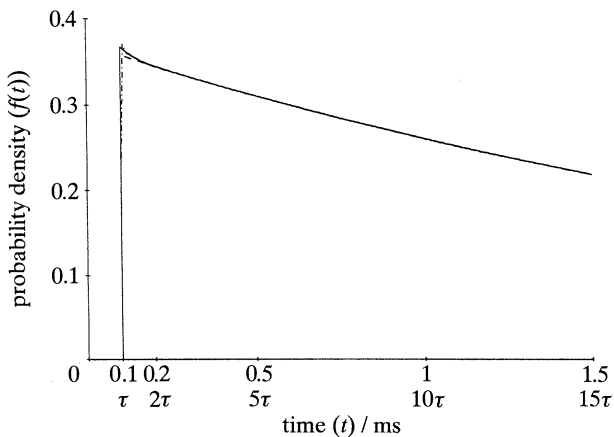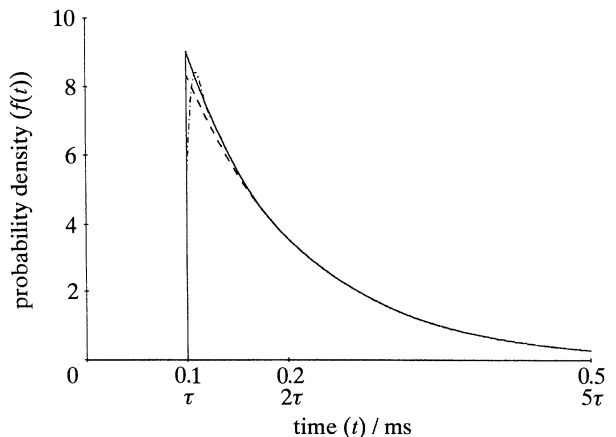Figure 7. Probability density of full e-shut times, $f_T(t)$, in model BM2, together with the first (dashed) and sixth (dot-dashed) asymptotic approximations.

Table 6. *Model BM2: shut times*

(Description as for table 1.)

component	1	2	3	4	5
μ/ms	0.11751	0.01565	0.01315	0.01196	0.01123
ω/(rad. ms^{-1})	0	44.495	114.65	180.33	244.75
area					
a	0.98112	−0.03140	−0.00727	−0.00330	−0.00190
b	0	0.03970	0.01790	0.01104	0.00785

t value range	maximum error (%)				
$0-\tau/4$	7.7	30	36	39	41
$\tau/4-\tau/2$	4.5	0.76	1.3	0.50	0.58
$\tau/2-3\tau/4$	2.0	0.36	0.12	0.053	0.32
$3\tau/4-\tau$	0.39	0.11	0.028	0.017	8.1×10^{-3}
$\tau-2\tau$	0.12	0.031	0.010	4.5×10^{-3}	2.4×10^{-3}
$2\tau-3\tau$	5.4×10^{-4}	1.9×10^{-5}	4.6×10^{-6}	1.4×10^{-6}	4.9×10^{-7}
$3\tau-4\tau$	7.2×10^{-7}	1.4×10^{-8}	1.5×10^{-9}	3.0×10^{-10}	8.5×10^{-11}

an average shut time (this is close to what would be found by fitting data that obeyed this model; see discussion at the beginning of § 4. On the other hand, the mean observed excess open time, $2.89 - 0.1 = 2.79$ ms, is nearly three times the mean open time (1 ms), and there are $E(R) = 2.718$ open times per e-open time. The density of e-open times is plotted in figure 6 and an analysis of the excess e-open distribution is given in table 5. The corresponding analysis for e-shut times is given in figure 7 and table 6. Note that, in the latter case, only four complex roots were found with the truncation point used. The slower process, the open times, have a more nearly exponential distribution (the area, a_1, for the first component in table 5 being very nearly 1).

(b) General observations

From these examples we note that slower processes,

relative to the dead-time τ, tend to be more nearly exponential. Also, in each example, we notice that the oscillating frequencies, and to a lesser extent the damping 'means' μ_n (except for the first, exponential, component), are very similar for both open and closed e-times. In all examples we have considered the simple exponential asymptotic approximation to the probability density of excess times, $f_{\tilde{T}}(t)$, appears sufficiently accurate for any practical purpose for (excess) t greater than τ, or at most 2τ, using a criterion of 1% accuracy. The complication of adding the damped oscillations is aimed at improving the fit for small t: however, it is precisely for small t that the exact density given by equations (19–24) is simple and accurate to compute.

Although the study of the damped oscillations has been interesting, we conclude that a sensible recipe for calculating these densities, $f_{\tilde{T}}(t)$, is to use the exponential for large t, the exact density for small t, with the change-over being made at some convenient point

where they agree to a sufficient accuracy, usually somewhere between τ and 2τ.

5. THE GENERAL CASE

We now consider the general case where there are more than two states. From equation (18) we see that the asymptotic behaviour of $^{\mathscr{A}}R(t)$, and hence of $f_{\tilde{T}}(t)$, depends on the values of s which render singular the matrix $W(s)$ defined as

$$W(s) = sI - H(s), \tag{52}$$

where

$$H(s) = Q_{\mathscr{A}\mathscr{A}} + Q_{\mathscr{A}\mathscr{F}} \left(\int_0^{\tau} e^{-st} \exp(Q_{\mathscr{F}\mathscr{F}}t) \, dt \right) Q_{\mathscr{F}\mathscr{A}}, \tag{53}$$

or, if s is not an eigenvalue of $Q_{\mathscr{F}\mathscr{F}}$ so that $(sI - Q_{\mathscr{F}\mathscr{F}})^{-1}$ exists,

$$H(s) = Q_{\mathscr{A}\mathscr{A}} + Q_{\mathscr{A}\mathscr{F}}(sI - Q_{\mathscr{F}\mathscr{F}})^{-1}(I - \exp(-(sI - Q_{\mathscr{F}\mathscr{F}})\tau)) Q_{\mathscr{F}\mathscr{A}}. \tag{54}$$

In other words, we are interested in the roots of the determinantal equation

$$\det W(s) = 0. \tag{55}$$

Jalali & Hawkes (1992b) prove several theorems, which we summarize here in the form of one composite theorem.

(a) Theorem

1. If $H(s)$ is irreducible (which follows if all states of the Markov chain intercommunicate), det $W(s) = 0$ has always a simple real root $s_1 < 0$ which is greater than the real part of any other root. Then s_1 is an eigenvalue of $H(s_1)$ and asymptotically

$$^{\mathscr{A}}R(t) \sim e^{s_1 t} c_1 r_1 / r_1 W'(s_1) c_1$$

where c_1, r_1 are the corresponding right (column) and left (row) eigenvectors and $W'(s_1)$ is the derivative with respect to s of the matrix $W(s)$ evaluated at the root s_1.

2. When Q is irreducible and reversible (see Kelly 1979), det $W(s) = 0$ has exactly $k_{\mathscr{A}}$ real roots. If these are distinct, then, as $t \to \infty$,

$$\mathscr{A}R(t) \sim \sum_{i=1}^{k_{\mathscr{A}}} e^{s_i t} c_i r_i / r_i W'(s_i) c_i$$

where c_i, r_i are the right and left eigenvectors of $H(s_i)$ corresponding to the root s_i, which is also an eigenvalue of $H(s_i)$.

The matrix derivative in the above results can be evaluated as

$$W'(s) = I + Q_{\mathscr{A}\mathscr{F}}[S_{\mathscr{F}\mathscr{F}}^*(s)(sI - Q_{\mathscr{F}\mathscr{F}})^{-1}$$
$$- \tau(I - S_{\mathscr{F}\mathscr{F}}^*(s)]G_{\mathscr{F}\mathscr{A}}^*(s), \quad (56)$$

where $S_{\mathscr{F}\mathscr{F}}^*(s)$ and $G_{\mathscr{F}\mathscr{A}}^*(s)$ are defined in equations (17) and (4), respectively.

As models of ion channels are always assumed to obey the principle of microscopic reversibility, in the absence of external energy supply (see Colquhoun & Hawkes 1982, pp. 24–25), the second part of the theorem is relevant. It follows from this theorem and equation (15) that the probability density of excess e-open times, $f_T(t)$, will be asymptotically represented as a linear combination of $k_{\mathscr{A}}$ negative exponential terms. This is attractive because this is the same form that the density has in the ideal case when no intervals are missed.

We shall therefore represent the asymptotic probability density of excess e-open times in the form

$$f_T(t) = \sum_{i=1}^{k_{\mathscr{A}}} a_i(1/\mu_i)\exp(-t/\mu_i), \quad (57)$$

where

mean $\mu_i = -1/s_i$,

area $a_i = \mu_i \phi_{\mathscr{A}} c_i r_i Q_{\mathscr{A}\mathscr{F}} \exp(Q_{\mathscr{F}\mathscr{F}} \tau) u_{\mathscr{F}} / r_i W'(s_i) c_i$. (58)

We conjecture that, as in the two-state case discussed in § 3, there will also be infinitely many complex conjugate pairs of roots. This, however, is rather more difficult to prove and the complex roots more difficult to find. We have found that in practice the approximation given by the real roots only is quite sufficient.

(b) Finding the roots and eigenvectors

To implement the above results in practice we need to be able to find the real roots and the corresponding eigenvectors. One can make a plot of det $W(s)$, calculated from equations (52) and (54) as a function of real s, and identify the roots approximately. Each of these can then be located precisely by a simple bisection method.

Once a root s has been found, the left eigenvalue, r, can be found as a solution to $rW(s) = 0$, $ru = 1$, (where u is a vector of ones). These are just like the equations for finding an equilibrium vector of a Q-matrix and can be solved in a similar manner, see, for example, Hawkes & Sykes (1990). The right eigenvector, c, can

be found in a similar way, as its transpose satisfies $c^T W^T(s) = 0$, $c^T u = 1$.

Alternatively, a Newton–Raphson method may be used to iterate simultaneously the root and the right eigenvector so that, if s_r, c_r are the rth iterates, the next iteration is given by

$$c_{r+1} = (u^T W^{-1}(s_r) W'(s_r) c_r)^{-1} W^{-1}(s_r) W'(s_r) c_r$$
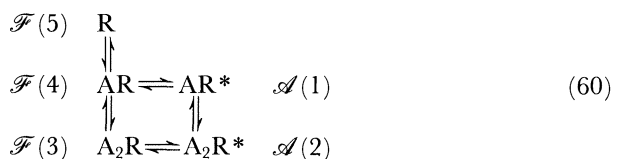$$s_{r+1} = s_r - (u^T W^{-1}(s_r) W'(s_r) c_r)^{-1}. \quad (59)$$

The left eigenvector can then be found as described above.

In general, Newton–Raphson procedures either work very well or they fail. We have found that it usually works well in this case, but the initial value of s sometimes needs to be very close to the true value for it to converge to the desired root.

6. NUMERICAL EXAMPLES WITH MORE THAN TWO STATES

In this section we study three examples with more than two states. To consider the effect of varying the dead-time τ, the standard model in each case uses τ equal to 0.2 ms and we also study a good resolution model with $\tau = 0.05$ ms and a poor resolution model with $\tau = 0.5$ ms, keeping all the rate constants fixed. Again, we use milliseconds as the unit of time throughout.

Model CH82. This model, discussed in Colquhoun & Hawkes (1982), 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 equation (60) and the matrix of rate constants (rates per millisecond) is shown partitioned in equation (61).

$$\begin{array}{ll}
\mathscr{F}(5) & R \\
& \updownarrow \\
\mathscr{F}(4) & AR \rightleftharpoons AR^* \quad \mathscr{A}(1) \\
& \updownarrow \qquad \updownarrow \\
\mathscr{F}(3) & A_2R \rightleftharpoons A_2R^* \quad \mathscr{A}(2)
\end{array} \quad (60)$$

The model defined in equations (60) and (61) 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 lifetime (100 ms) and channel activations are well-separated (by 3789 ms on average, see table 7a). The channel activations consist predominantly of several 'long' openings (each usually a single sojourn in state 2, mean life ≈ 2 ms, since direct transition from states 1 to 2 are rare), and these are separated by brief shuttings which consist mainly of single sojourns in state 3 (mean life $= 1/19 \approx 53$ μs, manifested as the large (73%) component of shut times with a time constant of 53 μs in table 7a. There are rare longer interruptions too (component with time constant 0.485 ms and 0.8% of area in table 7a). A few channel activations are brief single openings corresponding mainly to sojourns in state 1 (mean life $= 1/3.05 = 0.328$ ms).

$$Q = \begin{bmatrix} -3.05 & 0.05 & \vdots & 0 & 3 & 0 \\ 0.0006666667 & -0.5006666667 & \vdots & 0.5 & 0 & 0 \\ \cdots & \cdots & \cdots & \cdots & \cdots & \cdots \\ 0 & 15 & \vdots & -19 & 4 & 0 \\ 0.015 & 0 & \vdots & 0.05 & -2.065 & 2 \\ 0 & 0 & \vdots & 0 & 0.01 & -0.01 \end{bmatrix} \quad (61)$$

To investigate the distributions expected when brief events are missed, we begin by finding the asymptotic densities of observed lifetimes. For the standard model the exploratory plots of det $W(s)$ against s are shown in figure 8a,b. We can clearly see the two and three roots, respectively, for the open and shut cases. These were found more accurately by the Newton–Raphson method described in the previous section and the means and areas for the representation of equation (57) calculated from equation (58). These are shown in the top half of table 7a, together with similar results for other values of τ. When there is perfect resolution, $\tau = 0$, the representation as a mixture of exponentials is exact and the areas a_i sum to 1. There is no reason for the sum to be 1 in the general case, but it does turn out to be close.

The exact probability density for e-open times, $f_T(t)$, with $\tau = 0.2$ ms is shown in figure 8c together with the exponential asymptote and the mixture of two exponentials obtained by using both roots: the latter is almost indistinguishable from the exact density.

Similarly, the distribution of e-shut times with $\tau = 0.2$ ms is shown in figure 8d together with asymptotic densities making use of two, and three real roots. With all three exponentials the agreement with the exact PDF is good after one dead time, but in the first interval, $t = \tau$ to 2τ, the exponential approximation is too slow and too small to provide a good fit. The approximation is derived for large t, so this is not surprising, but the nature of the exact PDF in this first interval is interesting. From equation (22) it can be seen that in the first interval the exact PDF is simply a mixture of k exponentials, with time constants that are (minus the reciprocals of) the eigenvalues of Q (the zero eigenvalue corresponding to a constant term). This contrasts with the PDF when no events are missed ($\tau = 0$), which is a mixture of $k_{\mathscr{F}}$ exponentials with time constants derived from eigenvalues of $Q_{\mathscr{F}\mathscr{F}}$. In this case the fastest eigenvalue of both Q and of $Q_{\mathscr{F}\mathscr{F}}$ are similar, corresponding to time constants of 51.5 µs and 52.6 µs respectively (the diagonal element $q_{33} = -19$ ms^{-1}, $-1/q_{33} = 52.6$ µs, dominates both). Thus the exact PDF shows (the tail of) the fastest component of shut times quite accurately in this case despite the fact that the time constant is only about 25% of the dead time.

A summary of percentage errors, comparing the asymptotic densities with one, two or three components against the exact density, over various ranges of t (expressed as multiples of τ: note that these refer to excess times $\tilde{T} = T - \tau$) is given in the lower half of table 7a. We see how the accuracy increases by adding more components. The accuracy is very good indeed for the open-time distribution and good, except for small t, for the shut-time distribution.

When we consider different values of τ, we see from the error summaries in table 7a that the two-component asymptote remains a very accurate approximation to the open-time distribution even for $\tau = 0.5$ ms. The pictures of the densities are similar to figure 8c, and so have not been reproduced. In the case of shut times, the three component approximation is quite good when $\tau = 0.05$ ms, being almost indistinguishable from the exact density, and is therefore not illustrated. For the poor resolution case with $\tau = 0.5$ ms, see figure 8e, we find that the two-component approximation is above the exact density for small t so that, to improve on this, the third component gets a negative area. The three-component asymptote is, nevertheless, reasonably accurate for e-shut times greater than 2τ (excess times greater than τ).

Now consider the structure of the e-intervals and the individual lifetimes of which they are composed. The results, calculated by the methods of equations (25–31), are summarized in table 7b. We start with the shut times, which are more interesting. We see from the entry probabilities with $\tau = 0$ that most shut times start in state 3 and never in state 5, and the overall mean shut time is 993 ms. However, the first shut interval T_1 of an e-interval begins when a shut time has already been in progress for time τ, shifting the probabilities towards occupying the longer lived shut states (4,5) at that time: the distribution of T_1 is modified in a manner related to that expressed in Hawkes *et al.* (1990, equation 5.4). E(T_1) is increased in consequence, the more so for larger τ, reaching 3745 ms when $\tau = 0.5$ ms. As the average number of shut times per e-shut time, E(R), is quite small (at most 1.54 in this example) this is the major effect which τ has on the mean excess e-shut time, E($\tilde{T}$): it could be said that the critical dead-time for observing a shut time is more important than the dead-time for missing an open time.

It is interesting to note, however, that the mean duration of subsequent shut times, E(T_r), is also increased, but for quite different reasons. The second or subsequent shuttings of an e-shut time, where they exist, follow short openings and cannot start in state 5 but are rather more likely to start in state 4 (by a state 1 to state 4 transition). This also leads to increased mean shut times; we give here only the second one, E(T_2), and the limiting case of E(T_r) for large r (denoted E(T_∞)). Note that the mean lifetimes starting from a given state (see equation (29)), are shown at the foot of table 7b. The E(T_r) are obtained from weighting these by the appropriate entry probabilities (see equation (30)).

When considering open times, we see that the entry probabilities, and hence the means E(T_r), are not all that much affected by the dead-time: most openings begin by a state 3 to state 2 transition, especially after

Table 7. *Model CH82*

(Results concerning excess e-lifetimes, $\tilde{T} = T - \tau$, using various dead-times, τ. The top half of (a) contains the means and areas of the exponential components of the asymptotic probability densities, as given by equations (57) and (58). The bottom half of (a) shows the maximum absolute percent error of the asymptotic forms relative to the exact density, over various ranges of t: each successive asymptote is obtained by adding one extra component to the previous one. In (b) are the means and entry probabilities for individual lifetimes T_1, T_2, and T_r for large r (denoted T_∞) of an e-lifetime, together with $E(R)$, $E(\tilde{T})$, $E(T_{open})$ and $E(T_{shut})$. The means open and shut times starting from a given state are also shown. All times (τ, means) are in milliseconds.)

(a)

τ	components	open times 1	2	shut times 1	2	3
0	means	2.00	0.328	3789	0.485	0.053
	areas	0.928	0.072	0.262	0.008	0.730
0.05	means	3.89	0.328	3952	0.485	0.054
	areas	0.884	0.116	0.469	0.013	0.515
0.2	means	8.91	0.329	4387	0.487	0.079
	areas	0.841	0.159	0.920	0.018	0.046
0.5	means	9.74	0.331	5039	0.490	0.201
	areas	0.922	0.077	0.991	0.011	-0.002

τ	t value range	maximum error (%)				
0.05	$0-\tau$	61	0.20	100	99.7	1.7
	$\tau-2\tau$	58	1.4×10^{-4}	100	99.4	6.0×10^{-3}
	$2\tau-3\tau$	54	3.5×10^{-8}	100	98.6	8.2×10^{-6}
	$3\tau-4\tau$	51	2.9×10^{-9}	100	96.8	6.8×10^{-9}
	$4\tau-5\tau$	47	3.4×10^{-9}	100	93.0	6.3×10^{-9}
0.2	$0-\tau$	84	0.41	100	97	46
	$\tau-2\tau$	74	3.5×10^{-4}	99.7	65	3.9
	$2\tau-3\tau$	61	1.0×10^{-7}	98.9	19	0.107
	$3\tau-4\tau$	47	1.2×10^{-8}	98.1	2.7	6.6×10^{-4}
	$4\tau-5\tau$	33	1.1×10^{-8}	97.1	0.329	7.6×10^{-6}
0.5.	$0-\tau$	72	1.3	99	35	41
	$\tau-2\tau$	36	7.8×10^{-4}	97	12	0.94
	$2\tau-3\tau$	12	1.4×10^{-7}	93	2.5	0.049
	$3\tau-4\tau$	3.0	2.1×10^{-9}	84	0.50	2.7×10^{-3}
	$4\tau-5\tau$	0.71	4.6×10^{-10}	65	0.089	1.3×10^{-4}

(b)

	open times $E(R)$ $E(\tilde{T})$ $E(T_{open})$	r	$E(T_r)$	entry probabilities ϕ_1	ϕ_2	shut times $E(R)$ $E(\tilde{T})$ $E(T_{shut})$	r	$E(T_r)$	entry probabilities ϕ_3	ϕ_4	ϕ_5
τ											
0	1	1	1.88	0.0741	0.9259	1	1	993	0.9259	0.0741	0
0.05	1.83	1	1.80	0.1187	0.8813	1.04	1	1767	0.6610	0.3154	0.0236
	3.47	2	2.00	0.0003	0.9997	1855	2	2119	0.5393	0.4607	0
	3.46	∞	2.00	0.0001	0.9999	1855	∞	2373	0.4520	0.5480	0
0.2	3.84	1	1.73	0.1627	0.8373	1.22	1	3470	0.0840	0.6663	0.2497
	7.54	2	2.00	0.0011	0.9989	4035	2	2583	0.3800	0.6200	0
	7.41	∞	2.00	0.0005	0.9995	4035	∞	2163	0.5240	0.4760	0
0.5	4.49	1	1.87	0.0803	0.9197	1.54	1	3745	0.0015	0.4046	0.5939
	9.01	2	2.00	0.0017	0.9983	4994	2	2432	0.4317	0.5683	0
	8.83	∞	2.00	0.0012	0.9988	4994	∞	1797	0.8499	0.3501	0

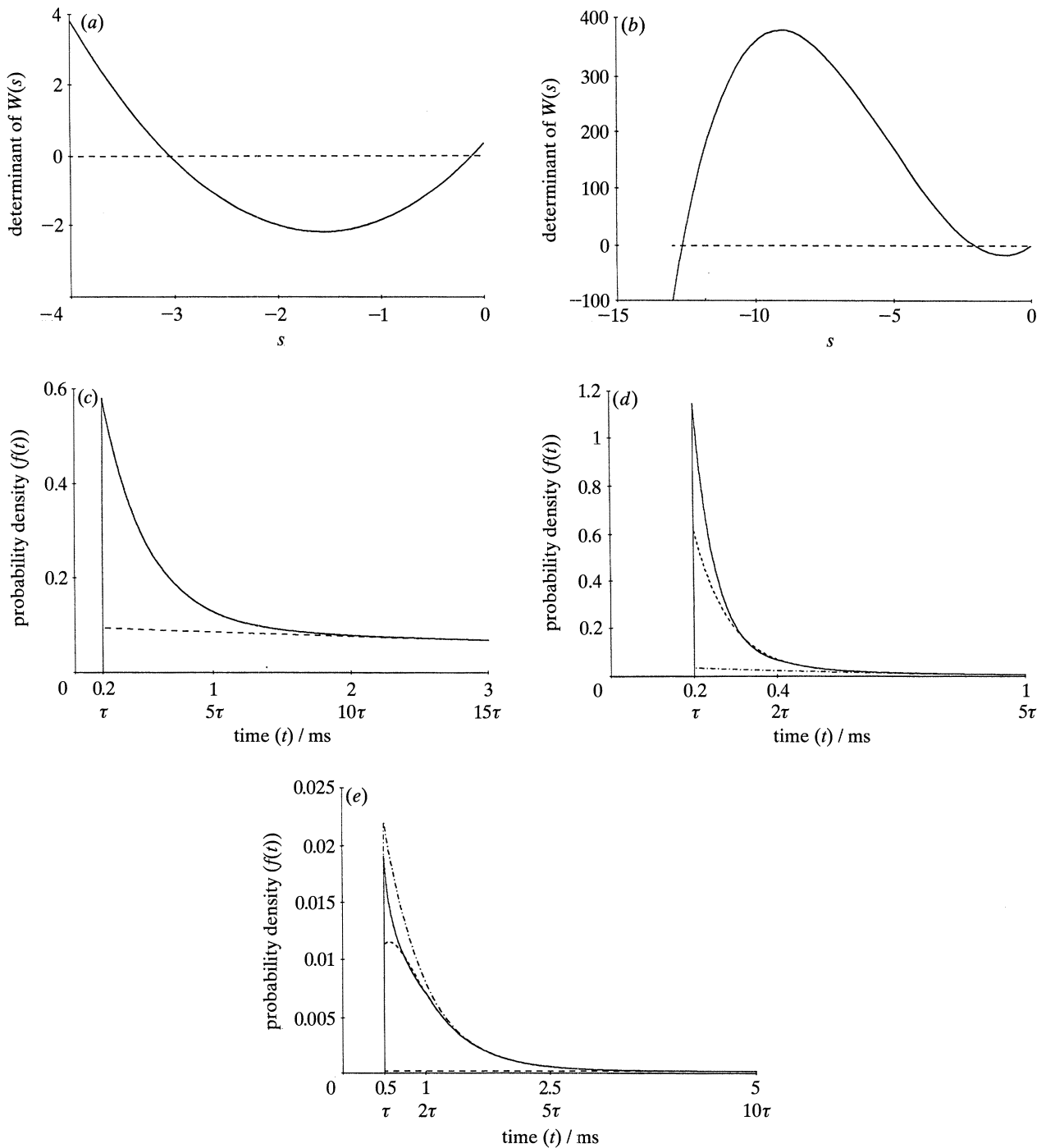| Mean open lifetimes starting from state		Mean shut lifetimes starting from state		
1	2	3	4	5
0.361	2.00	777	3690	3790

Figure 8. Plot of the determinant of $W(s)$ against s for (a) the open times and (b) the shut times arising from model CH82 with $\tau = 0.2$. These enable initial estimates of the two or three (respectively) zeros, i.e. the values of s for which det $W(s) = 0$. The remaining graphs show exact and asymptotic probability densities of observed lifetimes arising from model CH82. In each graph the exact density is shown by a solid line. In (c) is shown the open time density when $\tau = 0.2$; the single exponential asymptote is shown dashed whereas the double exponential asymptote is visually indistinguishable from the exact result. In (d) is shown the shut time density when $\tau = 0.2$, the double exponential asymptote (dot-dashed) and the treble exponential asymptote (short dashes). In (e) is shown the shut-time density when $\tau = 0.5$; the successive exponential asymptotes are shown long-dashed, dot-dashed and short dashed, respectively.

a short shut period. Consequently, the major effect on increasing $E(\tilde{T})$ is the concatenation effect of increasing numbers of openings contributing to an e-opening, with $E(R)$ reaching 4.49 when $\tau = 0.5$ ms.

Model CKF. Next consider a model with only one

open state and two shut states which was considered by Castillo & Katz (1957) and studied in connection with noise analysis by Colquhoun & Hawkes (1977) (where it was referred to as the 'KM scheme with full agonist'). The model, which may be thought of as a simplification of the previous model in which only one

agonist molecule can bind to the receptor, is illustrated in equation (62) and its transition rate matrix is given in equation (63).

$$R \rightleftharpoons AR \rightleftharpoons AR^* \tag{62}$$

state: $\mathscr{F}(3)$ $\mathscr{F}(2)$ $\mathscr{A}(1)$
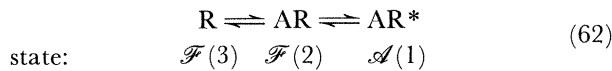
$$Q = \begin{bmatrix} -1 & \vdots & 1 & 0 \\ \cdots & \cdots & \cdots & \cdots \\ 19 & \vdots & -29 & 10 \\ 0 & \vdots & 0.026 & -0.026 \end{bmatrix} \tag{63}$$

The mean lives of the two shut states are 0.034 ms and 38 ms, one short and one very long compared with the dead-time. Consequently, the entry probabilities for the first shut time T_1 of an e-shut time vary considerably with the value of τ (see table 8*b*), and therefore have a big effect on the mean duration $E(T_1)$, as was the case in the previous example. From the Q-matrix we see that shut times normally begin in state 2 and the mean shut lifetime starting from that state is 20.3 ms (see table 8*b* with $\tau = 0$). However, a

Table 8. *Model CKF*

(Results on excess e-lifetimes, $\tilde{T} = T - \tau$, using various dead-times, τ. The top half of (*a*) contains the means and areas of the exponential components of the asymptotic probability densities, see equations (57) and (58). Below are the maximum absolute percent errors of the asymptotic forms relative to the exact density, over various ranges of *t*: successive asymptotes are obtained by adding one more component to the previous one. In (*b*) are the means $E(R)$, $E(T_1)$ and $E(\tilde{T})$ and entry probabilities for the start of an e-interval. Note that, for $r > 1$, $E(T_r)$ and the probabilities are the same as for the case $\tau = 0$. All times are in milliseconds.)

(*a*)

τ	components	open times	shut times	
		1	1	2
0	means	1	58.7	0.034
	areas	1	0.345	0.655
0.05	means	2.03	59.8	0.037
	areas	0.99993	0.697	0.297
0.2	means	2.95	63.2	0.077
	areas	0.99975	0.995	0.003
0.5	means	2.99	71.9	0.229
	areas	0.99970	1.001	−0.002

τ	*t* value range	maximum error (%)		
0.05	0–τ	0.97	100	5.6
	τ–2τ	9.8×10^{-4}	99	0.071
	2τ–3τ	3.6×10^{-7}	98	3.0×10^{-4}
	3τ–4τ	6.3×10^{-11}	92	2.4×10^{-7}
	4τ–5τ	2.4×10^{-13}	75	1.5×10^{-9}
0.2	0–τ	2.2	89	65
	τ–2τ	4.7×10^{-4}	15	1.8
	2τ–3τ	3.3×10^{-8}	1.3	0.020
	3τ–4τ	5.8×10^{-13}	0.095	4.3×10^{-4}
	4τ–5τ	2.0×10^{-13}	7.0×10^{-3}	8.7×19^{-6}
0.5	0–τ	2.3	35	30
	τ–2τ	4.7×10^{-5}	5.6	0.420
	2τ–3τ	9.7×10^{-10}	0.61	0.012
	3τ–4τ	1.9×10^{-13}	0.070	4.4×10^{-4}
	4τ–5τ	1.9×10^{-13}	8.0×10^{-3}	1.2×10^{-5}

(*b*)

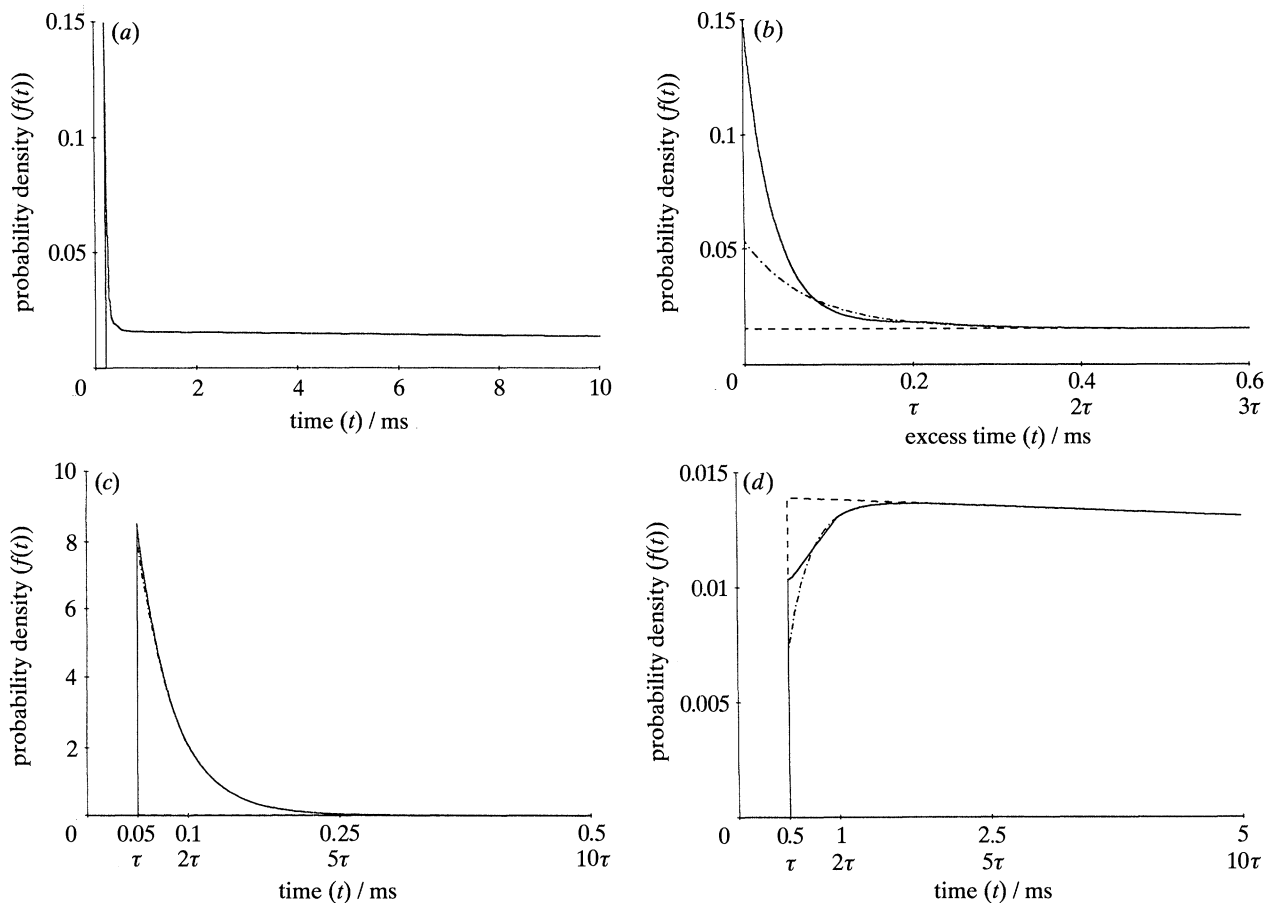|	open times			shut times			entry probabilities	
τ	$E(R)$	$E(T_1)$	$E(\tilde{T})$	$E(R)$	$E(T_1)$	$E(\tilde{T})$	ϕ_2	ϕ_3
0	1	1	1	1	20.3	20.3	1	0
0.05	2.01	1	2.03	1.05	40.6	41.7	0.471	0.529
0.2	2.89	1	2.95	1.22	58.4	62.9	0.010	0.990
0.5	2.92	1	2.99	1.65	71.9	72.0	0.001	0.999

Figure 9. Exact and asymptotic probability densities of observed shut times arising from model CKF. In each graph the exact density is shown by a solid line. In (a) is shown the density of observed shut times, $f_T(t)$, when $\tau = 0.2$. The sharp peak at the start is investigated in (b) by plotting the density of excess shut times, $f_{\tilde{T}}(t)$, for t in the range $0, 3\tau$). In (c) is shown the density of full e-shut times, $f_T(t)$ when $\tau = 0.05$; the two asymptotes are close to the horizontal axis and the exact curve, respectively. In (d) is shown the density of full e-shut times, $f_T(t)$, when $\tau = 0.5$. In each of (b), (c) and (d) the single exponential asymptote is shown dashed whereas the double exponential asymptote is shown dot-dashed.

shut time starting in state 3 takes $1/0.026 = 38.5$ ms on average (the mean lifetime of state 3) to reach state 2 and then a further 20.3 ms on average before the shut time terminates, so the average time starting from state 3 is 58.8 ms. Shifting the entry probabilities towards state 3, therefore, clearly has an effect. As $E(R)$ remains quite small, this is clearly the major contributor to the mean excess shut time, the remainder being contributed by subsequent shut times and short openings which make up the e-shut time. As there is only one open state, the remaining shut times all begin with a state 1 to state 2 transition and so, for $r > 1$, the T_r all have the same distribution as in the case $\tau = 0$.
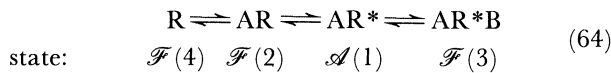
The above effect does not occur if there is only one state. The mean of any open lifetime (including $E(T_I)$) is therefore 1 ms, which is quite long, even compared with the poor resolution dead-time, and the duration of the e-open time is entirely dictated by the concatenation effect.

Turning now to the distribution of lifetimes, we note that the distribution of excess e-open times is very nearly exponential, as shown by the areas (which are very close to 1) and the percentage errors given in table 8a, and not worth graphing.

The exact density for e-shut times from the standard model ($\tau = 0.2$ ms) is shown in figure 9a. It has a very sharp peak near τ. Figure 9b examines this peak in more detail, plotting now the density of excess times, $f_{\tilde{T}}(t)$: the single exponential asymptote naturally misses the peak completely; adding the second component copes with the tail of it but is well short at the origin. This sharp peak is, in fact, a quite accurate representation of the fastest shut time component, for the reasons discussed above for the CH82 model (with $\tau = 0.2$ ms). In this case the fastest eigenvalue of Q corresponds to 33.7 µs, and the fastest eigenvalue of $Q_{\mathscr{F}\mathscr{F}}$ corresponds to 34.5 µs (the diagonal element $q_{22} = -29$ ms^{-1}, $-1/q_{22} = 34.5$ µs, dominates both); again the exact PDF represents (the tail of) the fastest shut time component quite accurately despite the fact that its time constant is only about 16% of the dead time.

In the good resolution case, figure 9c, adding the second component gives a good fit to nearly the whole distribution. The poor resolution case is very interesting. Figure 9d shows the density of e-shut times, showing a small depression, instead of a peak, at the beginning: the second component, which has a negative area, makes a reasonable attempt to follow it.

Model CB (channel block model). Our final model is obtained by adding one extra shut state to the previous model, by supposing that a blocking molecule, B, can enter and block the open channel. The model is illustrated in equation (64) and the matrix of transition rates is given in equation (65). Again the standard model assumes $\tau = 0.2$ ms. The model is similar to one discussed in Colquhoun & Hawkes (1990). The mean open time is 0.5 ms, the mean shut time, at 9.4 ms, is quite long but it includes a short mean blocking time of 0.02 ms. The other parameters are chosen so that the channel is open about 5% of the time.

$$R \rightleftharpoons AR \rightleftharpoons AR^* \rightleftharpoons AR^*B$$

state: $\quad \mathscr{F}(4) \quad \mathscr{F}(2) \quad \mathscr{A}(1) \quad \mathscr{F}(3) \qquad (64)$

$$Q = \begin{bmatrix} -2 & \vdots & 1 & 1 & 0 \\ \cdots & \cdots & \cdots & \cdots & \cdots \\ 1 & \vdots & -101 & 0 & 100 \\ 50 & \vdots & 0 & -50 & 0 \\ 0 & \vdots & 5.6 & 0 & -5.6 \end{bmatrix} \qquad (65)$$

For shut times, the variation in $E(T_1)$ with τ, shown in table 9b, is quite small (for $\tau \neq 0$), most e-shut times starting in state 4, so that the increase of $E(\tilde{T})$ with τ is mainly due to the concatenation effect of increasing $E(R)$. The mean duration of all shut times T_r, $r > 1$, is 9.4 ms (because there is only one open state from which the set of shut states can be entered). On the other hand, if an open time is interrupted by a short shut time then it is almost certainly a block of a brief sojourn in state 2 (although isolated sojourns in 2 are

Table 9. *Model CB*

(Description as for table 8.)

τ	components	open times	shut times		
		1	1	2	3
0	means	0.5	19.0	0.02	9.4×10^{-3}
	areas	1	0.496	0.5	0.004
0.05	means	0.948	20.0	0.024	9.4×10^{-3}
	areas	0.9998	0.927	0.065	-0.0001
0.2	means	1.04	23.7	0.077	9.5×10^{-3}
	areas	0.9995	1.001	-0.001	-4.2×10^{-13}
0.5	means	1.06	35.5	0.237	—
	areas	0.998	1.003	-0.003	—

τ	t value range		maximum error (%)		
0.05	$0-\tau$	1.5	99	21	21
	$\tau-2\tau$	1.4×10^{-3}	88	1.1	1.1
	$2\tau-3\tau$	4.9×10^{-7}	47	2.8×10^{-3}	2.7×10^{-3}
	$3\tau-4\tau$	8.7×10^{-11}	9.9	4.4×10^{-5}	4.8×10^{-5}
	$4\tau-5\tau$	9.6×10^{-11}	1.3	6.0×10^{-8}	4.1×10^{-8}
0.2	$0-\tau$	2.2	17	30	30
	$\tau-2\tau$	1.3×10^{-4}	3.0	0.23	0.23
	$2\tau-3\tau$	5.5×10^{-9}	0.21	4.7×10^{-3}	4.7×10^{-3}
	$3\tau-4\tau$	3.7×10^{-12}	0.016	1.0×10^{-4}	1.0×10^{-4}
	$4\tau-5\tau$	3.4×10^{-12}	1.2×10^{-3}	2.2×10^{-6}	2.2×10^{-6}
0.5	$0-\tau$	2.9	46	29	—
	$\tau-2\tau$	1.5×10^{-3}	6.4	0.39	—
	$2\tau-3\tau$	8.4×10^{-7}	0.76	0.013	—
	$3\tau-4\tau$	2.4×10^{-10}	0.094	2.8×10^{-4}	—
	$4\tau-5\tau$	3.4×10^{-10}	0.011	3.7×10^{-6}	—

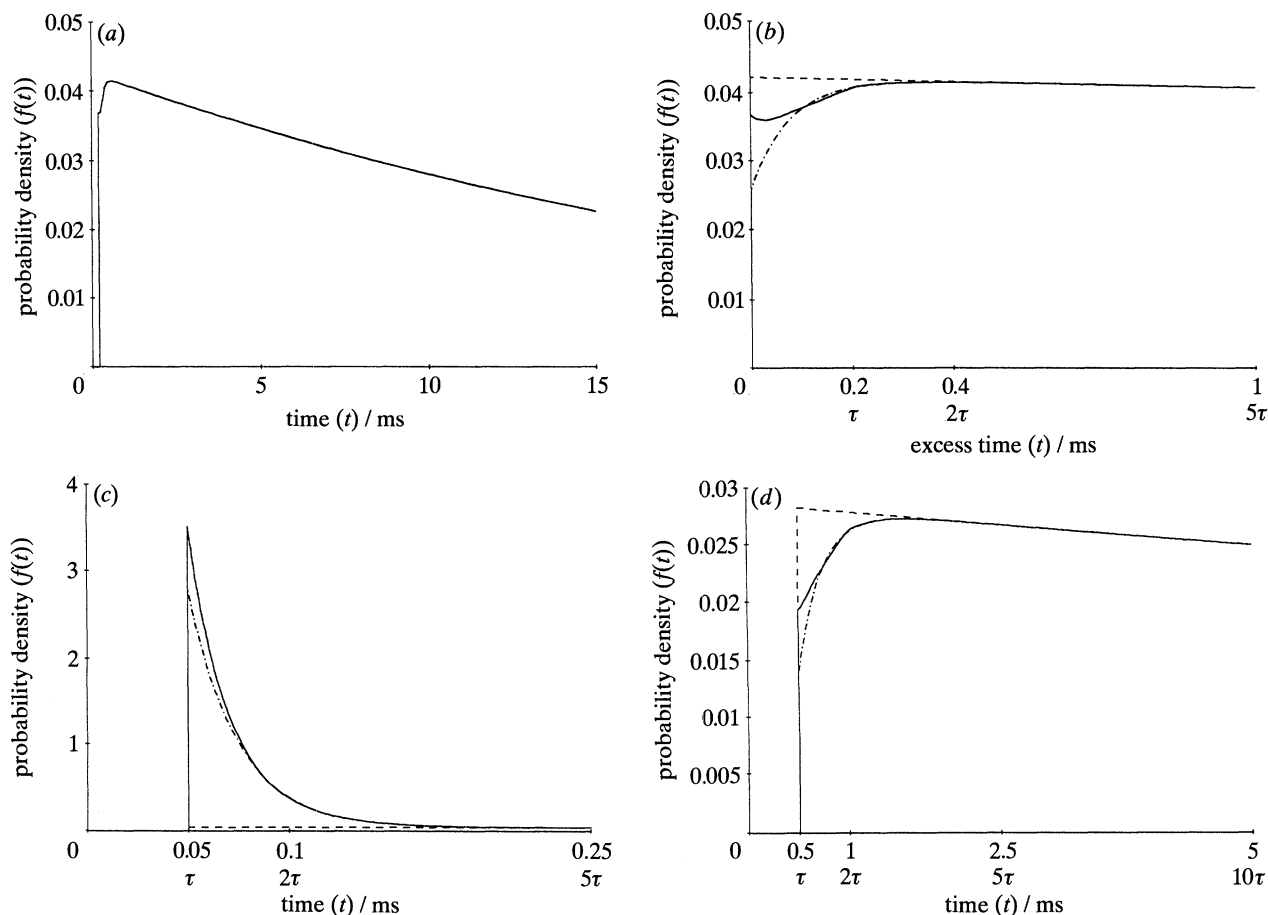|	open times			shut times			entry probabilities		
τ	$E(R)$	$E(T_1)$	$E(\tilde{T})$	$E(R)$	$E(T_1)$	$E(\tilde{T})$	ϕ_2	ϕ_3	ϕ_4
0	1	0.5	0.5	1	9.4	9.4	0.5	0.5	0
0.05	1.87	0.5	0.947	1.11	17.6	18.6	0.053	0.077	0.871
0.2	2.04	0.5	1.041	1.49	19.0	23.7	0.053	4.6×10^{-5}	0.947
0.5	2.07	0.5	1.063	2.72	19.0	35.6	0.053	1.4×10^{-11}	0.947

Figure 10. Exact and asymptotic probability densities of observed shut times arising from model CB. In each graph the exact density is shown by a solid line. In (a) is shown the density of observed shut times, $f_T(t)$, when $\tau = 0.2$. The dip at the start is investigated in (b) by plotting the density of excess shut times, $f_T(t)$, for t in the range $(0,5\tau)$. In (c) and (d) are shown the densities of full e-shut times, $f_T(t)$, when $\tau = 0.05$ and 0.5, respectively. In each of (b), (c) and (d) the single exponential asymptote is shown dashed whereas the double exponential asymptote is shown dot-dashed.

rare because once in 2 it is much more likely that the next transition is to 4, rather than re-opening to 1). These shut times are so short that most of them will be missed even at $\tau = 0.05$ ms, so making the resolution worse than this does not extend the mean e-open time much more. The PDF of e-open times is nearly exponential (areas close to 1 in table 9a) and not worth graphing.

The density of e-shut times shown in figure 10a has a dip near τ like the last example. This dip is shown in more detail in figure 10b where the excess shut time density, $f_T(t)$ together with the first and second asymptotic approximations, is plotted. Again, the second component has a negative area and succeeds in following the first part of the dip. The area of the third component is so small that it makes virtually no difference to the result.

In the case of good resolution the density of e-shut times, $f_T(t)$, shown in figure 10c, appears more regular. The third component has little effect here either. The situation with poor resolution, shown in figure 10d, is similar to that for the standard model. This time, however, the third component was not found because the calculation of det $W(s)$ for very negative s took us outside the range of accuracy obtainable with our software (Dyalog APL on an

IBMP70 with 80386 chip); we imagine it would have an area even smaller in magnitude than the 10^{-13} found when $\tau = 0.2$ ms (see table 9a). The ineffectiveness of, or in the last case failure to find, the third component is presumably a consequence of the shut-time distribution having a very short (about 0.01 ms) component with a small area, even with perfect resolution ($\tau = 0$ in table 9a).

7. COMPARISON OF ROUGH CORRECTIONS WITH THE EXACT RESULTS

The results given above provide a way to calculate the exact distributions in the presence of missed events, to a very close approximation. However the use of these results in practice is still limited by two main problems: (i) the results are for one channel, but it is often not known with any certainty how many channels are contributing to the recording; (ii) to use the results it is necessary (as with all other approximations; see Introduction) to specify a mechanism for the channel operation, which may not always be practicable (especially as the mechanism must include any desensitized states that may be present). These problems should be somewhat ameliorated when we

extend the results to the analysis of bursts of openings (in the manner of Colquhoun & Hawkes (1982)).

Some much cruder methods for correction for missed events, which do not encounter either of these difficulties, have been proposed (e.g. Colquhoun & Sakmann 1985; Ogden & Colquhoun 1985). The results in this paper provide an opportunity to test these methods.

If there were only one open-time and one shut-time component (which is virtually never true) then the mean open and shut times could be corrected as described by Colquhoun & Sigworth (1983), a method that was derived from the results presented here. In practical cases it is possible to make corrections without postulating a mechanism only if (virtually) all openings are long enough to be resolved, and only short shut periods are missed (or vice versa). In this case the openings will appear too long (because some shuttings are missed), but the shut time distribution will be correct apart from the fact that observations below $t = \tau$ are missing. For example the CH82 model has true ($\tau = 0$) shut-time time constants of 3789 ms, 0.485 ms and 53 μs, and with a resolution of $\tau = 0.05$ ms these are little changed (3952 ms, 0.485 ms and 54 μs; see table 7a). At first sight the areas of these components seem to be quite different when $\tau = 0.05$ ms, but this is largely because in this case the areas sum (almost) to unity over the range $t = \tau$ to ∞, whereas when $\tau = 0$ the areas sum to unity over $t = 0$ to ∞. If experimental results with $\tau = 0.05$ were fitted (above $t = 0.05$ or 0.1) with three exponential components (as discussed at the beginning of § 4), the areas would be scaled to sum to 1 over $t = 0$ to ∞. The result of the fitting would be similar to that shown in table 7a for $\tau = 0.05$ except that the areas given there must be renormalized by multiplying each area by $\exp(\tau/\mu_i)$, (where the μ_i are the time constants of the shut time distribution), and then rescaled to sum to 1. When this is done the areas for $\tau = 0.05$ become 0.263, 0.008, 0.729, very similar to those for $\tau = 0$ (0.262, 0.008, 0.730). This shows that fitting shut time data should give a good approximation to the true shut time distribution if few openings are missed (this will, of course, not be the case with the open time distribution because many shuttings are missed in this example). This shut time distribution will be written here as

$$f(t) = \sum a_i (1/\mu_i) \exp(-t/\mu_i). \quad (66)$$

(a) Case where there is a single component open-time distribution and only shuttings are missed

The proportion of shut times that are shorter than τ is

$$P(T \leqslant \tau) = \int_0^\tau f(t) dt = 1 - \sum a_i \exp(-\tau/\mu_i), \quad (67)$$

and the mean length of such gaps is

$$\mu_{sg} = \frac{\int_0^\tau t f(t) dt}{P(T \leqslant \tau)}$$

$$= \frac{\sum a_i \{\mu_i - (\mu_i + \tau)\exp(-\tau/\mu_i)\}}{1 - \sum a_i \exp(-\tau/\mu_i)}. \quad (68)$$

Similarly the mean length of gaps longer than τ, is given by

$$\mu_{lg} = \frac{\int_\tau^\infty t f(t) dt}{P(T > \tau)} = \tau + \frac{\sum a_i \mu_i \exp(-\tau/\mu_i)}{\sum a_i \exp(-\tau/\mu_i)}, \quad (69)$$

where

$$P(T > \tau) = \int_\tau^\infty f(t) \, dt = \sum a_i \exp(-\tau/\mu_i). \quad (70)$$

The mean number of openings per e-opening, $E(R)$, is

$$E(R) \approx 1/\sum a_i \exp(-\tau/\mu_i), \quad (71)$$

and the mean total shut time per e-opening is

$$\mu_{TS} = (E(R) - 1)\mu_{sg}. \quad (72)$$

If the fitted length of an e-opening is denoted μ_{co}, the apparent mean open time (adjusted by subtracting τ to allow for observing only openings longer than τ), the mean total open time per e-opening, $E(T_{open})$ can be found as

$$E(T_{open}) = \mu_{co} - \mu_{TS}, \quad (73)$$

so the corrected mean open time is

$$\mu_O = E(T_{open})/E(R). \quad (74)$$

These arguments can be applied to models BM2 and CKF. For model BM2 the mean excess e-open and e-shut times were 2.79 ms and 0.116 ms respectively and these should be close to what would be found by fitting data. These values give 2.37 openings per e-opening (from equation (71)), and a corrected mean open time of 1.15 ms (from equation (74)). The true values are 2.72 ms and 1.0 ms respectively; the correction would be worthwhile in practice, though the 16% error in the mean shut time gives rise to a similar error in the results.

For model CKF, with $\tau = 0.05$ ms, the fitted open time would be about 2.03 ms (table 8), and the fitted shut time have time constants close to 59.8 ms and 37 μs, with areas (after renormalization of the values in table 8 as described above) of 0.380, 0.620 respectively (not too far from the true, $\tau = 0$, values of 0.345, 0.655). These values give 1.85 openings per e-opening (from equation (71)), and a corrected mean open time of 1.09 ms (from equation (74)). The true values are 2.01 ms (table 8b) and 1.0 ms respectively.

In these two examples a useful correction is obtained when the resolution is good, but if the resolution is much worse than this the results become inadequate.

(b) Case where there is a two-component open-time distribution, only shuttings are missed, and 'short openings' have no gaps

When the open-time distribution has two components, as in the CH82 model, there is no way of telling, when the mechanism is unspecified, whether

missed shuttings are missed from the 'long openings' or the 'short openings'. A rough correction can still be made if we are willing to assume that the 'short openings' in fact contain no gaps, so there are none to be missed and 'short e-openings' are therefore the same as true 'short openings'. A similar argument was used by Colquhoun & Sakmann (1985).

It is, of course, quite improper, in general, to refer to the components of the open time distribution as 'short openings' and 'long openings', as though they were physically distinct entities. Nevertheless, under some circumstances, this nomenclature, although not precise, does make physical and mathematical sense (see, for example, Colquhoun & Hawkes 1982; Colquhoun & Sakmann 1985).

Say N_o = total number of openings, and N_e = total number of e-openings in the channel record, so

$$N_o/N_e \approx \mathrm{E}(R) \tag{75}$$

from equation (71). Define also a_s and a_f as the areas of the slow and fast components of the distribution of (true) openings, and a_s', a_f' similarly for the distribution of e-openings (both over 0 to ∞). Suppose too, *ex hypothesi*, that the number and duration of 'short openings' is the same as the number and duration of 'short e-openings' so $N_o a_f \approx N_e a_f'$. Thus

$$\mathrm{E}(R) \approx \frac{N_o(a_f + a_s)}{N_e(a_f' + a_s')} \approx \frac{N_e a_f' + N_o s_s}{N_e},$$

so

$$a_s \approx 1 - a_f' \sum a_i \exp(-\tau/\mu_i). \tag{76}$$

This gives an estimate of a_s, and hence $a_f = 1 - a_s$, for the 'true open times'. The corrected mean open times can be estimated as follows. The 'short open times' have mean μ_f as for the e-open times (*ex hypothesi*). The corrected mean length of a 'long opening', μ_{lo}, can be found thus

$\mu_{lo} =$

$$\frac{\text{length of long e-opening} - \text{shut time in long e-opening}}{\text{number of openings per long e-opening}}. \tag{77}$$

The number of openings per long e-opening, $\mathrm{E}(R_1)$ say, is

$$\mathrm{E}(R_1) = \frac{\text{number of openings in long e-openings}}{\text{number of long e-openings}},$$

$$= \frac{\begin{array}{c}\text{total number of openings} - \\ \text{(number of openings in short e-openings)}\end{array}}{\text{number of long e-openings}},$$

$$= \frac{\text{total number of openings} - \text{number of short e-openings}}{\text{number of long e-openings}},$$

$$= \frac{N_o - N_e a_f'}{N_e a_s'} \approx \frac{\mathrm{E}(R) - a_f'}{a_s'}. \tag{78}$$

The shut time per long e-opening is, since all missed gaps are, *ex hypothesi*, missed from long openings, just the number of (missed) gaps per long e-opening multiplied by the mean length of a missed gap from equation (68), namely

$$(\mathrm{E}(R_1) - 1)\mu_{sg} \tag{79}$$

This completes everything that is needed for evaluation of equation (77).

These results can be exemplified by the CH82 model. The renormalization of the shut time distribution in this case was discussed above (before equation (66)), and it was clear that fitting shut time data would give a good estimate of the true time distribution in the case where $\tau = 0.05$ ms. In this case the open time distribution has two components and so it must be similarly renormalized; this converts the areas of 0.884 and 0.116 (for $\tau = 0.05$ ms in table 7a) to 0.869 and 0.131 respectively; these are taken as a_s', a_f', and the time constant for the latter, 3.89 ms, is taken as the mean length of a 'long e-opening' for equation (77). The overall number of openings per e-opening is estimated (from equation (71)) to be 1.79 (true value 1.83, from table 7b), and the overall corrected mean open time is 1.91 ms (true value 1.88 ms). The mean number of openings per 'long e-opening', from equation (78), comes to 1.91. The corrected open-time distribution gives time constants of 2.03 ms (from equation (77)), and 0.328 ms (as observed, *ex hypothesi*), with areas (a_s and a_f) of 0.927 and 0.073, respectively. These are quite close to the true ($\tau = 0$) open-time distribution (time constants 2.00 ms and 0.328 ms, with areas 0.928 and 0.072; table 7a), despite the grossness of the assumptions that were made.

8. DISCUSSION

We have found that computation of the exact probability density of observed open (or shut) times affected by the omission of short intervals, obtained in Hawkes *et al.* (1990), is generally quite feasible, both in time and accuracy, for up to about 20 times the dead-time. However, it gets steadily more complicated and time consuming as t increases and ultimately the series becomes numerically unstable. In this paper we have studied several examples which show that the asymptotic form of $f_T(t)$ for excess open or closed lifetimes, consisting of a linear combination of exponentials, is simple and very accurate for t greater than the dead-time τ or at most 2τ (i.e. $t > 2\tau$, or at most, 3τ for the full observed lifetimes, $T = \tilde{T} + \tau$), and sometimes for smaller t. If the processes are slow compared with the dead-time, the asymptotic form may be a very good approximation to the exact density for all t.

Thus we have the best of both worlds: a simple exact density for small t, and a relatively simple and very accurate asymptote for larger t. One may simply change over from one to the other when satisfied that the asymptotic approximation has come close enough. This is true even in the two-state case, where we found it is possible to improve the single (in that case) exponential asymptote by adding damped oscillations. These complicate the issue only to improve the approximation over a range of t where the exact result is easy to calculate anyway: we think it is better to use only the exponential terms.

With more than one open or shut state we have found that, if there are states with mean lifetimes which are short compared to the dead-time, the

distribution of excess lifetimes can have depressions near the origin and, in consequence, some exponential components in the asymptotic form have negative areas. If there are mean lifetimes which are very short, not all of the $k_{\mathscr{A}}$ components may be effective.

The mean observed shut time, say, is naturally increased by concatenation if short open times in the middle are missed. However, if the mean lives of the various shut states differ widely, the increase may also be partly due to changes in the initial probabilities of the states in which an e-shut period may begin.

We have discussed this in terms of the distribution of observed lifetimes because it is of practical interest and simple to look at. What we have really done, however, is to find a relatively simple way of computing $^{\mathscr{A}}R(t)$, which is central to computing many things. Using this, for example, it is feasible to compute the likelihood for the complete observed process, as discussed in section six of Hawkes et al. (1990). We have used partly graphical methods to find the roots but the process can be more-or-less completely automated, thus facilitating the exploration of the likelihood surface. This has recently been implemented, and the results will be presented elsewhere.

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