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A note on correlations in single ion channel records

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General expressions are derived for the correlation coefficients between the length of an opening and that of the nth subsequent opening for a single ion channel. Analogous results are given for the correlation between shut times, and between an open time and subsequent shut times. An alternative derivation of the results of Fredkin *et al.* (in *Proc. Berkeley Conf. in honor of Neyman & Kiefer*, vol. 1, pp. 269–289 (1985)) is given, and their results are extended to the case where openings occur in bursts. Expressions are given for the correlation between the first and nth opening in a burst, between the lengths of bursts, and between the number of openings per burst. Each of these sorts of correlation can give information about the connections that exist between the various states of the system; interpretations of the correlations are discussed.

Expressions are derived for the distributions of the nth open time, shut time, burst length, etc. following the application of a perturbation (e.g. a voltage jump or a concentration jump). It is shown that these distributions will all be the same (namely the equilibrium distribution) only in the case where the openings, burst lengths, etc. are not correlated.

Certain reaction schemes predict a component in the distribution of the number of openings per burst that has a unit mean (i.e. a component of isolated single openings). For some schemes this component is predicted to have zero amplitude, in principle, whereas in others it may be quite prominent. The presence or absence of this component can give information about the way in which the various states of the system are connected. The interpretation in terms of mechanism is discussed.

0. INTRODUCTION

The interpretation of observations of single ion channel currents has, as one of its major goals, the establishment of a qualitative reaction mechanism for the opening and shutting of the ion channel. Once this has been established it will then usually be possible to estimate rate constants for at least some of the transitions that are involved in the mechanism. It has recently been shown by Fredkin *et al.* (1985) that observations on correlations between successive open times can give important information concerning the number of routes by which the various states of the system can interconvert. Such measurements have been used as an aid to interpretation of experimental results by Jackson *et al.* (1983), Labarca *et al.* (1985), McManus *et al.* (1985) and Colquhoun & Sakmann (1985).

It is our aim in this paper (*a*) to provide explicit general equations by means of which the magnitude of correlations that are predicted by any specific mechanism may be calculated, for comparison with experimental measurements, (*b*) to provide an alternative proof of the theorem of Fredkin *et al.* (1985) concerning the decay of such correlations, (*c*) to extend the results of Fredkin *et al.* (1985) to correlations within and between bursts of openings and (*d*) to discuss the distributions to be expected after a perturbation (e.g. a voltage jump), the form of which depends on the presence or absence of correlations.

We shall also discuss the inferences that can be made from the presence or absence of a component with near-unit mean in the distribution of the number of openings per burst. The observation of such a component can give information about the connections between states which is different from, though related to, that inferred from correlations.

1. General principles

We shall assume throughout that the reaction mechanism can be described as a Markov process (Colquhoun & Hawkes 1977, 1981, 1982, 1983; Fredkin *et al.* 1985).

The origin of correlations

The Markov assumption implies that if the system is in a specified state at time t, the future evolution of the system is independent of what happened before t. The lifetimes of sojourns in individual states are therefore independent of each other, and are thus uncorrelated. Correlations can, however, arise in the experimental observations when, as is usually the case, it is not possible to distinguish all of the individual states of the system by looking at the record.

Consider, for example, scheme 1 in figure 1. This scheme has three shut states that are experimentally indistinguishable, and two open states that are supposed to have equal conductance so that they too are experimentally indistinguishable. The experimental record would show only whether the channel was 'open' (in either of the open states) or 'shut' (in one of the three shut states). Suppose further that the mean lifetime of sojourns in open state 1 is shorter than that in open state 2, and that transitions between shut states 3 and 4, and between open states 1 and 2, are rather slow. In this case an opening that starts with a $3 \rightarrow 2$ transition is likely to be followed by several more $2 \rightarrow 3 \rightarrow 2$ transitions (so several 'long' openings would occur in succession), before a $3 \rightarrow 4$ transition. Once in state 4 there would then be several $4 \rightarrow 1 \rightarrow 4$ transitions, which would give rise to several 'short' openings in succession. Thus a short opening would tend to be followed by another short opening (and a long opening by another long opening), so there would be a positive correlation between successive open times. If there were few $2 \rightarrow 3 \rightarrow 2$ and $4 \rightarrow 1 \rightarrow 4$ oscillations (e.g. if $3 \rightleftharpoons 4$ transitions were rapid) there would be little correlation between open times; the correlation arises essentially from 'bursting' behaviour. It may be noted here that two separate and independent channels with different bursting characteristics may give rise to correlations, even when neither alone would show correlations.

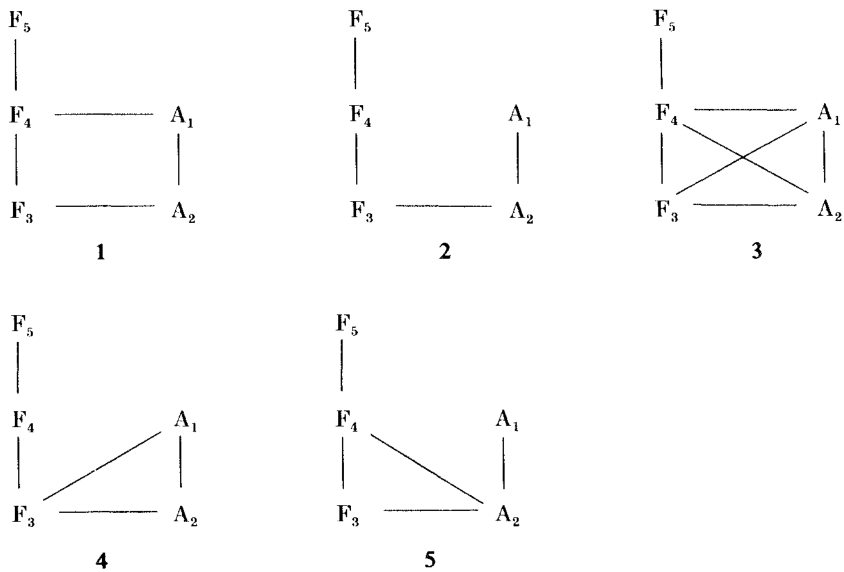


FIGURE 1. Examples of possible connections between open ($\mathscr{A}$) states and shut ($\mathscr{F}$) states (see text). The number of each individual state is shown as a subscript. A line joining two states indicates that reversible transitions between the states are possible. Note that the existence or nonexistence of connections *within* $\mathscr{A}$ states, and *within* $\mathscr{F}$ states, is irrelevant to the determination of the form of correlations; only connections *between* $\mathscr{A}$ and $\mathscr{F}$ matter.

Correlations can arise only if there are at least two experimentally indistinguishable shut states and two indistinguishable open states. As Fredkin *et al.* (1985) pointed out, the appearance of a correlation will depend on the routes that exist for transitions between states. For example, scheme **2** in figure 1 is the same as **1** except for the routes between states. But no correlations can occur in **2** because *every* opening must start with the same ($3 \rightarrow 2$) transition; what happens after this transition must be independent of what happened before it.

Notation

The notation used here will be the same as that in Colquhoun & Hawkes (1982), to which reference should be made for details.

The k states in which the system can exist will be divided into a subset $\mathscr{A}$ that contains $k_{\mathscr{A}}$ open states, and a subset $\mathscr{F}$ that contains the remaining $k_{\mathscr{F}}$ shut states. The individual open states (the members of the set $\mathscr{A}$) will be denoted by roman letters as A_1, A_2, etc., the individual open states being distinguished by the subscripts. Similar notation is used for the members of other sets. For the analysis of bursts of openings the shut states will be divided further into subsets $\mathscr{B}$ (short-lived shut states, $k_{\mathscr{B}}$ in number, that constitute gaps within bursts), and $\mathscr{C}$, which contain longer lived shut states ($k_{\mathscr{C}}$ in number) such that any entry into $\mathscr{C}$ is deemed to end the burst and hence to generate a gap between bursts. Thus $\mathscr{F} = \mathscr{B} \cup \mathscr{C}$ and $k_{\mathscr{F}} = k_{\mathscr{B}} + k_{\mathscr{C}}$. Finally, we define the subset $\mathscr{E}$, which contains both $\mathscr{A}$ and $\mathscr{B}$ states, the states in which the system resides during a burst of openings, so $\mathscr{E} = \mathscr{A} \cup \mathscr{B}$ contains $k_{\mathscr{E}} = k_{\mathscr{A}} + k_{\mathscr{B}}$ states.

The matrix of transition rates between states will be denoted $\boldsymbol{Q}$, and partitioned sections of it as $\boldsymbol{Q}_{\mathscr{A}\mathscr{A}}$, $\boldsymbol{Q}_{\mathscr{A}\mathscr{F}}$, etc. Expressions of the following type (which are discussed fully by Colquhoun & Hawkes 1982) will occur frequently.

$$\boldsymbol{P}_{\mathscr{A}\mathscr{A}}(t) = \mathrm{e}^{\boldsymbol{Q}_{\mathscr{A}\mathscr{A}}t}, \tag{1.1}$$

its Laplace transform

$$\boldsymbol{P}^*_{\mathscr{A}\mathscr{A}}(s) = (s\boldsymbol{I}-\boldsymbol{Q}_{\mathscr{A}\mathscr{A}})^{-1}, \tag{1.2}$$

$$\boldsymbol{G}^*_{\mathscr{A}\mathscr{F}}(s) = \boldsymbol{P}^*_{\mathscr{A}\mathscr{A}}(s)\,\boldsymbol{Q}_{\mathscr{A}\mathscr{F}}, \tag{1.3}$$

and the transition probability matrix $\boldsymbol{G}^*_{\mathscr{A}\mathscr{F}}(0)$ for transitions from open to shut states, which will be denoted simply as $\boldsymbol{G}_{\mathscr{A}\mathscr{F}}$, given by

$$\boldsymbol{G}_{\mathscr{A}\mathscr{F}} = -\boldsymbol{Q}^{-1}_{\mathscr{A}\mathscr{A}}\,\boldsymbol{Q}_{\mathscr{A}\mathscr{F}}. \tag{1.4}$$

Rank and spectral expansion

The following definitions are given, for example, in Mirsky (1955). The rank of a matrix is the number of linearly independent rows (or columns) that it contains; alternatively, the rank is the maximum value of r for which there exists (after permutation of rows and columns if necessary) an $r \times r$ submatrix with a non-zero determinant. The rank of any matrix $\boldsymbol{X}$ will be denoted $R(\boldsymbol{X})$. If $\boldsymbol{X}$ is a $k \times k$ matrix then $k - R(\boldsymbol{X})$ is called the nullity of $\boldsymbol{X}$. The number of zero eigenvalues that $\boldsymbol{X}$ has will usually be equal to its nullity (particular values of the rate constants could give more zero eigenvalues, but such particular cases are not of great interest in practice). Thus a non-singular matrix (i.e. one with a non-zero determinant) must have full rank ($R(\boldsymbol{X}) = k$, nullity = zero). Even when there are several zero eigenvalues, the conventional spectral expansion,

$$\boldsymbol{X}^n = \sum_{m=1}^{k} \boldsymbol{A}_m \lambda_m^n \tag{1.5}$$

will usually be valid. Here the λ_m represent the eigenvalues of $\boldsymbol{X}$, and the matrices $\boldsymbol{A}_m$ can be calculated from the eigenvectors of $\boldsymbol{X}$ as

$$\boldsymbol{A}_m = \boldsymbol{c}_m \boldsymbol{r}_m, \quad m = 1, 2, \ldots, k, \tag{1.6}$$

where $\boldsymbol{c}_m$ is the column eigenvector of $\boldsymbol{X}$ defined by $(\lambda_m \boldsymbol{I} - \boldsymbol{X})\,\boldsymbol{c}_m = \boldsymbol{0}$ and $\boldsymbol{r}_m$ is the row eigenvector of $\boldsymbol{X}$ defined by $\boldsymbol{r}_m(\lambda_m \boldsymbol{I} - \boldsymbol{X}) = \boldsymbol{0}$. The eigenvectors are scaled so that the matrix with $\boldsymbol{r}_m$ as its rows is the inverse of the matrix with $\boldsymbol{c}_m$ as its columns so that $\Sigma\,\boldsymbol{A}_m = \boldsymbol{I}$. The $\boldsymbol{A}$ matrices also have the following properties (see, for example, Colquhoun & Hawkes 1977).

$$\boldsymbol{A}_i^n = \boldsymbol{A}_i, \quad \text{and} \quad \boldsymbol{A}_i \boldsymbol{A}_j = \boldsymbol{0}, \quad i \neq j. \tag{1.7}$$

Some frequently used transition matrices

It will be convenient to define here, for later use, three frequently used probability transition matrices. First, we define as $\boldsymbol{X}_{\mathscr{A}\mathscr{A}}$ the probability transition matrix that represents transitions from the start of one opening to the start of the next opening, namely

$$\boldsymbol{X}_{\mathscr{A}\mathscr{A}} = \boldsymbol{G}_{\mathscr{A}\mathscr{F}}\,\boldsymbol{G}_{\mathscr{F}\mathscr{A}}. \tag{1.8}$$

The i,jth element of $\boldsymbol{X}_{\mathscr{A}\mathscr{A}}$ gives the probability, given that an opening starts in open state i, that, after oscillation among the open states, followed by shutting and subsequent oscillation among the shut states, the channel eventually reopens to the open state j. Since the channel must eventually reach one of the open states the row sums of $\boldsymbol{X}_{\mathscr{A}\mathscr{A}}$ (like those of $\boldsymbol{G}_{\mathscr{A}\mathscr{F}}$ and of $\boldsymbol{G}_{\mathscr{F}\mathscr{A}}$) are unity, so

$$\boldsymbol{X}_{\mathscr{A}\mathscr{A}}\boldsymbol{u}_{\mathscr{A}} = \boldsymbol{u}_{\mathscr{A}}, \quad \boldsymbol{G}_{\mathscr{A}\mathscr{F}}\boldsymbol{u}_{\mathscr{F}} = \boldsymbol{u}_{\mathscr{A}}, \quad \boldsymbol{G}_{\mathscr{F}\mathscr{A}}\boldsymbol{u}_{\mathscr{A}} = \boldsymbol{u}_{\mathscr{F}}, \tag{1.9}$$

where $\boldsymbol{u}_{\mathscr{A}}$ represents a $(\boldsymbol{k}_{\mathscr{A}} \times 1)$ column vector with unit elements.

Secondly, we may define a matrix, $\boldsymbol{H}_{\mathscr{A}\mathscr{A}}$, that describes transitions from the start of an opening to the start of the next opening *in the same burst*, namely

$$\boldsymbol{H}_{\mathscr{A}\mathscr{A}} = \boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}}. \tag{1.10}$$

Unlike the other cases this will not have unit row sums because it does not describe all possible routes from the start of one opening to the start of the next; it excludes routes via the $\mathscr{C}$ states.

Thirdly, we may similarly define (as in Colquhoun & Hawkes 1982, equations 3.88 and 5.5–5.7), for channels that show bursting behaviour, a matrix for transition from the start of an opening to the first arrival (possibly via $\mathscr{B}$) in a $\mathscr{C}$ state, namely

$$\boldsymbol{G}_{\mathscr{A}(\mathscr{B})\mathscr{C}} = (\boldsymbol{I}-\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{-1}(\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{C}}+\boldsymbol{G}_{\mathscr{A}\mathscr{C}}), \tag{1.11}$$

and, for the transition from the start of a sojourn in $\mathscr{C}$ to the next opening, we define

$$\boldsymbol{G}_{\mathscr{C}(\mathscr{B})\mathscr{A}} = (\boldsymbol{I}-\boldsymbol{G}_{\mathscr{C}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{C}})^{-1}(\boldsymbol{G}_{\mathscr{C}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}}+\boldsymbol{G}_{\mathscr{C}\mathscr{A}}). \tag{1.12}$$

Because a gap between bursts is characterized by at least one sojourn in $\mathscr{C}$, we can define an analogue of (1.8) that describes transitions from the start of one burst of openings to the start of the next burst as

$$\begin{aligned}\boldsymbol{Z}_{\mathscr{A}\mathscr{A}} &= \boldsymbol{G}_{\mathscr{A}(\mathscr{B})\mathscr{C}}\boldsymbol{G}_{\mathscr{C}(\mathscr{B})\mathscr{A}},\\ &= (\boldsymbol{I}-\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{-1}(\boldsymbol{G}_{\mathscr{A}\mathscr{F}}\boldsymbol{G}_{\mathscr{F}\mathscr{A}}-\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}}),\\ &= (\boldsymbol{G}_{\mathscr{E}\mathscr{C}})_{\mathscr{A}\mathscr{C}}(\boldsymbol{G}_{\mathscr{F}\mathscr{A}})_{\mathscr{C}\mathscr{A}}.\end{aligned} \tag{1.13}$$

The first of these definitions follows directly from the descriptions of the routes from the start of one burst to the start of the next in (1.11) and (1.12), the second version follows from the results in Colquhoun & Hawkes (1982). The third version refers to the $\mathscr{A}\mathscr{C}$ subsection (i.e. the first $k_{\mathscr{A}}$ rows) of $\boldsymbol{G}_{\mathscr{E}\mathscr{C}}$, and the $\mathscr{C}\mathscr{A}$ subsection (i.e. the last $k_{\mathscr{C}}$ rows) of $\boldsymbol{G}_{\mathscr{F}\mathscr{A}}$, and describes in an intuitively elegant way the transition from burst ($\mathscr{E}$) states, starting in $\mathscr{A}$, to $\mathscr{C}$ states followed by transition from shut states ($\mathscr{F}$), starting in $\mathscr{C}$, back to $\mathscr{A}$.

The matrices in (1.11)–(1.13) all have, by a similar argument to that used above for $\boldsymbol{X}_{\mathscr{A}\mathscr{A}}$, unit row sums, i.e.

$$\boldsymbol{Z}_{\mathscr{A}\mathscr{A}}\boldsymbol{u}_{\mathscr{A}} = \boldsymbol{u}_{\mathscr{A}}, \quad \boldsymbol{G}_{\mathscr{A}(\mathscr{B})\mathscr{C}}\boldsymbol{u}_{\mathscr{C}} = \boldsymbol{u}_{\mathscr{A}}, \quad \boldsymbol{G}_{\mathscr{C}(\mathscr{B})\mathscr{A}}\boldsymbol{u}_{\mathscr{A}} = \boldsymbol{u}_{\mathscr{C}}. \tag{1.14}$$

Rank, the number of routes between subsets, and connectivity

Open and shut subsets

Consider $\boldsymbol{X}_{\mathscr{A}\mathscr{A}} = \boldsymbol{Q}_{\mathscr{A}\mathscr{A}}^{-1}\boldsymbol{Q}_{\mathscr{A}\mathscr{F}}\boldsymbol{Q}_{\mathscr{F}\mathscr{F}}^{-1}\boldsymbol{Q}_{\mathscr{F}\mathscr{A}}$, which is the probability transition matrix for transition from open states, via shut states, back to open. The rank of

$\boldsymbol{G}_{\mathcal{A}\mathcal{F}}$ will be the same as that for $\boldsymbol{Q}_{\mathcal{A}\mathcal{F}}$ (because the former is found by multiplying the latter by the non-singular matrix $-\boldsymbol{Q}_{\mathcal{A}\mathcal{A}}^{-1}$). The ranks of $\boldsymbol{Q}_{\mathcal{A}\mathcal{F}}$ and $\boldsymbol{Q}_{\mathcal{F}\mathcal{A}}$ (which describe the same $\mathcal{A}$–$\mathcal{F}$ routes but in opposite directions) will usually be equal, and $\boldsymbol{X}_{\mathcal{A}\mathcal{A}}$ will usually also have this same rank. The former assertion can be illustrated by considering the following hypothetical example which could apply to the scheme **3** in figure 1. The numbers on the borders of the matrix represent the state numbers, as shown in figure 1

$$\boldsymbol{Q}_{\mathcal{A}\mathcal{F}} = \begin{array}{c} \\ 1 \\ 2 \end{array}\!\!\begin{array}{c} \begin{array}{ccc} 3 & 4 & 5 \end{array} \\ \begin{bmatrix} 200 & 10 & 0 \\ 10 & 5 & 0 \end{bmatrix} \end{array}, \quad \boldsymbol{Q}_{\mathcal{F}\mathcal{A}} = \begin{array}{c} \\ 3 \\ 4 \\ 5 \end{array}\!\!\begin{array}{c} \begin{array}{cc} 1 & 2 \end{array} \\ \begin{bmatrix} 30 & 15 \\ 60 & 100 \\ 0 & 0 \end{bmatrix} \end{array}. \tag{1.15}$$

In this case both have rank 2, which is the maximum possible value, $k_{\mathcal{A}}$ (because the rank of a matrix cannot be larger than the number of rows or columns, whichever is the least). Particular numerical values could cause exceptions to this rule. If the top left-hand element in $\boldsymbol{Q}_{\mathcal{A}\mathcal{F}}$ was 20 rather than 200 then the first row of $\boldsymbol{Q}_{\mathcal{A}\mathcal{F}}$ would be exactly twice the second row (and the determinant of the leftmost 2×2 section of it would be zero) so the rank of $\boldsymbol{Q}_{\mathcal{A}\mathcal{F}}$ would be reduced to 1. Such numerical coincidences are never likely to be exactly true in physical reality (though they could, of course, be approximately true). The term *usually* is used here to indicate what will happen if the possibility of such numerical coincidences is neglected.

It may also be noted that in $\boldsymbol{Q}_{\mathcal{A}\mathcal{F}}$ in (1.15) there is a column of zeros because shut state 5 cannot communicate directly with either of the open states (figure 1*c*). There is a corresponding row of zeros in $\boldsymbol{Q}_{\mathcal{F}\mathcal{A}}$. In general, rows or columns of zeros will appear in $\boldsymbol{Q}_{\mathcal{F}\mathcal{A}}$ in the same position that they would in the transpose of $\boldsymbol{Q}_{\mathcal{A}\mathcal{F}}$, and the presence of a row or column of zeros will reduce the rank of a matrix by one. (The rank of $\boldsymbol{Q}_{\mathcal{A}\mathcal{F}}$ in (1.10) cannot be larger than $k_{\mathcal{A}} = 2$, so as long as it has two non-zero rows or columns it will usually have this maximum rank.) For example, the scheme **2** in figure 1, in which the only shutting route is $2 \rightarrow 3$, might have

$$\boldsymbol{Q}_{\mathcal{A}\mathcal{F}} = \begin{array}{c} \\ 1 \\ 2 \end{array}\!\!\begin{array}{c} \begin{array}{ccc} 3 & 4 & 5 \end{array} \\ \begin{bmatrix} 0 & 0 & 0 \\ 30 & 0 & 0 \end{bmatrix} \end{array}. \tag{1.16}$$

This has two zero columns and one non-zero column so its rank is 1. The same can be concluded from the fact that it has a zero row. Put another way, the rank can usually be found as the minimum number of rows (and/or columns) that must be deleted to leave only zeros undeleted; e.g. the first two columns in $\boldsymbol{Q}_{\mathcal{A}\mathcal{F}}$ in (1.15), and the bottom row (or the leftmost column) in (1.16).

The fact that (1.15) has rank 2 evidently corresponds to the fact that both of the two open states can communicate directly with different shut states in scheme **3** of figure 1 (as is also true in scheme **1**), whereas (1.16) has rank 1 because there is only one route from open to shut states in scheme **2**. In scheme **4** both open states communicate with $\mathcal{F}$, but they both communicate with the *same* shut state (state 3) so $\boldsymbol{Q}_{\mathcal{A}\mathcal{F}}$ has rank 1. In scheme **5** $R(\boldsymbol{Q}_{\mathcal{A}\mathcal{F}}) = 1$ also, for similar reasons. In both cases the channel must pass through a single state *en route* from $\mathcal{A}$ to $\mathcal{F}$.

Generalization of such arguments leads to the following conclusions (Fredkin *et al.* 1985). The rank of $\boldsymbol{Q}_{\mathscr{A}\mathscr{F}}$, $\boldsymbol{Q}_{\mathscr{F}\mathscr{A}}$ and of $\boldsymbol{X}_{\mathscr{A}\mathscr{A}}$, will usually be equal to whichever is the lesser of the following: (*a*) the number of different open states via which it is possible to leave $\mathscr{A}$ for the shut states ($\mathscr{F}$), (*b*) the number of different shut states via which it is possible to leave $\mathscr{F}$ for the open states ($\mathscr{A}$). Let this number be denoted C. Thus $R(\boldsymbol{X}_{\mathscr{A}\mathscr{A}}) \leqslant C$ (usually the equality sign will hold). For example we have $C = \min(2,2) = 2$ in **1** (figure 1); $C = \min(1,1) = 1$ in **2**; $C = \min(2,2) = 2$ in **3**; $C = \min(2,1) = 1$ in **4** and $C = \min(1,2) = 1$ in **5**. When $R(\boldsymbol{X}_{\mathscr{A}\mathscr{A}}) = 1$ there will be no correlation between open times (see Fredkin *et al.* 1985, and below). The number C can also be defined as the minimum number of states that must be deleted in order to separate completely the open states from the shut states (so that when the corresponding rows and/or columns are deleted from $\boldsymbol{Q}_{\mathscr{A}\mathscr{F}}$, only zeros remain, as described above).

Relation to graph theory

The term *state* corresponds to the term *vertex* (or *node*) as used in graph theory, and the term *connection* (i.e. a route for transition between two states) corresponds to *edge* in graph theory. The discussion above can now be made more rigorous (see, for example, Tainiter 1975). Regard $\mathscr{A}$, $\mathscr{F}$ as sets of vertices of a graph. Define the *vertex connectivity of* $\mathscr{A}$ *and* $\mathscr{F}$ as the minimum number of vertices whose removal disconnects $\mathscr{A}$ from $\mathscr{F}$ (note that removal of a vertex implies removal of the edges that are incident upon it). Denote this number by $C^{\mathrm{v}}(\mathscr{A},\mathscr{F})$; it is clearly the same as the number C defined above, and will be referred to hereinafter simply as the *connectivity*. We may also define (for use, particularly, in §7) a *gateway state between* $\mathscr{A}$ *and* $\mathscr{F}$ as any vertex (state) whose removal reduces $C^{\mathrm{v}}(\mathscr{A},\mathscr{F})$. Clearly the number of gateway states will often be greater than the connectivity. For example, in **1** and **3** the connectivity, $C^{\mathrm{v}}(\mathscr{A},\mathscr{F}) = 2$ (and the rank of $\boldsymbol{X}_{\mathscr{A}\mathscr{A}}$ will usually be 2), but states 1, 2, 3 and 4 are all gateway states. In **2**, **4** and **5** the connectivity (and the rank of $\boldsymbol{X}_{\mathscr{A}\mathscr{A}}$) is unity, but in **2** there are two gateway states (states 2 and 3), whereas in **4** there is only one gateway state (state 3), as is also the case in **5** (state 2).

Burst mechanisms

In this case the shut states are subdivided into short-lived shut states (subset $\mathscr{B}$) and long-lived shut states (subset $\mathscr{C}$). Arguments similar to those used above can be applied to $\boldsymbol{H}_{\mathscr{A}\mathscr{A}} = \boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}}$, which describe routes from $\mathscr{A}$ to $\mathscr{B}$ and back. The rank of $\boldsymbol{H}_{\mathscr{A}\mathscr{A}}$ will usually be the same as that of $\boldsymbol{Q}_{\mathscr{A}\mathscr{B}}$, and of $\boldsymbol{Q}_{\mathscr{B}\mathscr{A}}$. The connectivity between $\mathscr{A}$ and $\mathscr{B}$, $C^{\mathrm{v}}(\mathscr{A},\mathscr{B})$, can be defined exactly as above, as can a gateway state between $\mathscr{A}$ and $\mathscr{B}$. Note, however, that this definition will include indirect routes (via $\mathscr{C}$ states) between $\mathscr{A}$ and $\mathscr{B}$. For example, in scheme **20** of figure 2 there is no direct route (see below) between $\mathscr{A}$ and $\mathscr{C}$, but $C^{\mathrm{v}}(\mathscr{A},\mathscr{C}) = 1$ because there is an indirect route via state B_4 (which is the one $\mathscr{A}$–$\mathscr{C}$ gateway state in this case). This definition will be useful in the interpretation of correlations between bursts because the rank of $\boldsymbol{Z}_{\mathscr{A}\mathscr{A}}$ cannot be greater than, and will usually be equal to, $C^{\mathrm{v}}(\mathscr{A},\mathscr{C})$ (see (1.13) and §4).

In other cases, however, we will be interested only in *direct* routes between two

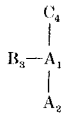
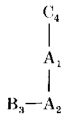
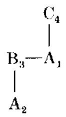
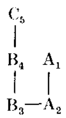
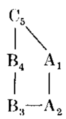
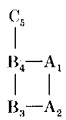
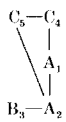
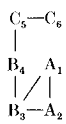
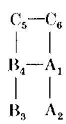
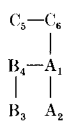
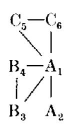
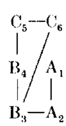
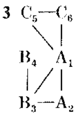
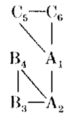
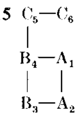
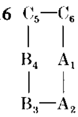
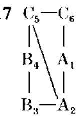
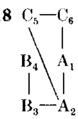
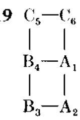
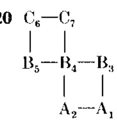
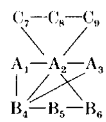
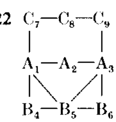
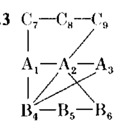
	scheme	rank Q_{AC}	rank Q_{BC}	rank X_{AA}	rank H_{AA}	rank Z_{AA}	area for $\mu = 1$
1		1	0	1	1	1	0
2		1	0	2	1	1	*
3		1	0	2	1	1	*
4		0	1	1	1	1	0
5		1	1	2	1	1	*
6		0	1	2	2	1	–
7		2	0	2	1	2	*
8		0	1	1	1	1	0
9		1	1	1	1	1	0
10		1	0	1	1	1	0
11		1	0	1	1	1	0
12		0	2	1	1	1	0
13		1	0	2	1	1	*
14		1	0	2	1	1	*
15		0	1	2	2	1	—
16		1	1	2	1	2	*
17		2	1	2	1	2	*
18		2	0	2	1	2	*
19		1	1	2	2	2	—
20		0	2	2	2	1	-
21		1	0	2	2	1	0
22		2	0	2	2	2	0
23		2	0	3	2	2	*

FIGURE 2. For description see opposite.

subsets of states. For example, when considering correlations within bursts we are interested only in direct transitions between $\mathscr{A}$ and $\mathscr{B}$ (any entry into a $\mathscr{C}$ state would signal the end of the burst). We therefore define the *direct vertex connectivity of $\mathscr{A}$ and $\mathscr{B}$*, as the minimum number of states deletion of which removes all direct connections between $\mathscr{A}$ and $\mathscr{B}$ (so the states removed belong to either $\mathscr{A}$ or $\mathscr{B}$, but not $\mathscr{C}$). This number will be denoted $D^{\mathrm{v}}(\mathscr{A},\mathscr{B})$, and will be referred to hereafter as the direct connectivity between $\mathscr{A}$ and $\mathscr{B}$. It will usually be the same as the rank of $\boldsymbol{H}_{\mathscr{A}\mathscr{A}}$. Similarly a *direct gateway state*† *between $\mathscr{A}$ and $\mathscr{B}$* can be defined as a state the removal of which reduces $D^{\mathrm{v}}(\mathscr{A},\mathscr{B})$. For example, schemes **1** and **3** in figure 2 both have $D^{\mathrm{v}}(\mathscr{A},\mathscr{B}) = 1$ (which is the rank of $\boldsymbol{H}_{\mathscr{A}\mathscr{A}}$); in scheme **1** states 1 and 3 are both direct $\mathscr{A}$–$\mathscr{B}$ gateway states, but in scheme **3** only state 3 is a direct $\mathscr{A}$–$\mathscr{B}$ gateway state. A more complex example is provided by schemes **22** and **23** in figure 2; the direct $\mathscr{A}$–$\mathscr{B}$ connectivity (and, usually, the rank of $\boldsymbol{H}_{\mathscr{A}\mathscr{A}}$) is two in both schemes, but the direct $\mathscr{A}$–$\mathscr{B}$ gateway states are states 1 and 3 in scheme **22**, and states 2, 4 and 6 in scheme **23**.

Two general results

The rank of the product of *any* two matrices, $\boldsymbol{X}$ and $\boldsymbol{Y}$ say, obeys the relation

$$R(\boldsymbol{XY}) \leqslant \min[R(\boldsymbol{X}), R(\boldsymbol{Y})]. \tag{1.17}$$

In the cases above, in which $\boldsymbol{X}$ and $\boldsymbol{Y}$ described the same routes (but in opposite directions) the equality sign will usually be correct in (1.17), but this will not

† In terms of graph theory we would define a *minimal $\mathscr{A}$–$\mathscr{B}$ vertex cutset* as a set of vertices, $C^{\mathrm{v}}(\mathscr{A},\mathscr{B})$ in number, deletion of which disconnects $\mathscr{A}$ and $\mathscr{B}$; so a gateway state between $\mathscr{A}$ and $\mathscr{B}$ is a vertex which belongs to some minimal $\mathscr{A}$–$\mathscr{B}$ vertex cutset. Similarly, a *direct minimal $\mathscr{A}$–$\mathscr{B}$ vertex cutset* is a set of vertices, $D^{\mathrm{v}}(\mathscr{A},\mathscr{B})$ in number, deletion of which removes *direct* connections between $\mathscr{A}$ and $\mathscr{B}$, and a direct gateway state between $\mathscr{A}$ and $\mathscr{B}$ is a one that belongs to some such cutset. For example, in figure 2, scheme **20**, $D^{\mathrm{v}}(\mathscr{A},\mathscr{B}) = 2 = R(\boldsymbol{H}_{\mathscr{A}\mathscr{A}})$, and the minimal direct $\mathscr{A}$–$\mathscr{B}$ vertex cutsets are $\{A_1, A_2\}$, $\{A_1, B_4\}$, $\{A_2, B_3\}$ and $\{B_3, B_4\}$, so A_1, A_2, B_3 and B_4 are all direct gateway states between $\mathscr{A}$ and $\mathscr{B}$.

FIGURE 2. A selection of hypothetical mechanisms for which openings occur in bursts. There is, of course, no suggestion that the more exotic schemes describe any real ion channel; they are shown merely to illustrate the principles in the text. The individual states are denoted A, B, C; they are the members of subsets, $\mathscr{A}$ (open), $\mathscr{B}$ (brief shut) and $\mathscr{C}$ (long-lived shut), respectively. Individual states are given numbers (shown as subscripts), for reference in the text.

The ranks of various probability transition matrices are shown. The ranks of $\boldsymbol{Q}_{\mathscr{A}\mathscr{C}}$ and $\boldsymbol{Q}_{\mathscr{B}\mathscr{C}}$ indicate the direct connectivity between $\mathscr{A}$ and $\mathscr{C}$, and between $\mathscr{B}$ and $\mathscr{C}$, respectively. The rank of $\boldsymbol{H}_{\mathscr{A}\mathscr{A}}$ is the same as that of $\boldsymbol{Q}_{\mathscr{A}\mathscr{B}}$, and the rank of $\boldsymbol{X}_{\mathscr{A}\mathscr{A}}$ is the same as that of $\boldsymbol{Q}_{\mathscr{A}\mathscr{F}}$. When the rank of $\boldsymbol{X}_{\mathscr{A}\mathscr{A}}$, $\boldsymbol{H}_{\mathscr{A}\mathscr{A}}$ or $\boldsymbol{Z}_{\mathscr{A}\mathscr{A}}$ is unity there will be no correlations respectively, between openings, within bursts, and between bursts.

The last column shows whether or not a component with unit mean is expected to be present in the distribution of the number of openings per burst. The symbol 0 indicates the absence of such a component (i.e. cases where it has zero area), * indicates its presence, and – indicates that there is no such component (which is the case when $R(\boldsymbol{H}_{\mathscr{A}\mathscr{A}}) = k_{\mathscr{A}}$ so $\boldsymbol{H}_{\mathscr{A}\mathscr{A}}$ has no zero eigenvalues).

The schemes are all shown with connections between the individual states *within* each set (e.g. between the individual open states), but the presence or absence of such connections does not affect whether or not correlations, or a finite area (last column), will be seen.

always be true in other cases (see, for example, the case discussed at the end of §4). Other general results are

$$\left.\begin{aligned} R(\boldsymbol{X}+\boldsymbol{Y}) &\leqslant R(\boldsymbol{X})+R(\boldsymbol{Y}), \\ R(\boldsymbol{X}-\boldsymbol{Y}) &\geqslant |R(\boldsymbol{X})-R(\boldsymbol{Y})|, \end{aligned}\right\} \tag{1.18}$$

but we cannot say that the equality sign in (1.18) will usually be correct in any of the cases that occur in this work.

Definition of the autocorrelation coefficient

The correlation coefficient for any quantities x_1, x_2 is defined as

$$\left.\begin{aligned} \rho(x_1, x_2) &= \frac{\operatorname{cov}(x_1, x_2)}{[\operatorname{var}(x_1)\operatorname{var}(x_2)]^{\frac{1}{2}}} \\ &= \frac{E(x_1 x_2)-\mu_1\mu_2}{\{[E(x_1^2)-\mu_1^2][E(x_2^2)-\mu_2^2]\}^{\frac{1}{2}}}, \end{aligned}\right\} \tag{1.19}$$

where we define the means as

$$\mu_1 = E(x_1), \quad \mu_2 = E(x_2).$$

When x_1 and x_2 have the same mean (μ, say) and variance (σ^2, say)

$$\rho(x_1, x_2) = [E(x_1 x_2)-\mu^2]/\sigma^2. \tag{1.20}$$

The expectations that are needed for the evaluation of this expression can be found from the Laplace transform, $f^*(s_1, s_2)$, of the joint distribution of x_1, x_2 from the general expression

$$E(x_1^n x_2^m) = (-1)^{n+m}\, \partial_{n+m} f^*(s_1, s_2)/\partial s_1^n\, \partial s_2^m|_{s_1=s_2=0}. \tag{1.21}$$

The following particular results will be needed frequently

$$-\mathrm{d}(s\boldsymbol{I}-\boldsymbol{Q}_{\mathscr{A}\mathscr{A}})^{-1}/\mathrm{d}s|_{s=0} = \boldsymbol{Q}_{\mathscr{A}\mathscr{A}}^{-2}, \tag{1.22}$$

and

$$\mathrm{d}^2(s\boldsymbol{I}-\boldsymbol{Q}_{\mathscr{A}\mathscr{A}})^{-1}/\mathrm{d}s^2|_{s=0} = 2\boldsymbol{Q}_{\mathscr{A}\mathscr{A}}^{-3}. \tag{1.23}$$

Calculation of equilibrium occupancies

The probability that the system is in each of its k states at equilibrium (the fraction in each state at equilibrium) will be denoted

$$\boldsymbol{p}(\infty) = [p_1(\infty) \quad p_2(\infty) \quad \dots \quad p_k(\infty)]. \tag{1.24}$$

For any specified reaction scheme these occupancies can easily be found by inspection, from the law of mass action. For the purposes of writing a general computer program it is convenient to have a general method of finding $\boldsymbol{p}(\infty)$ directly from any specified $\boldsymbol{Q}$ matrix; in any such algorithm care must be taken to avoid division by rate constants that happen to be zero. A method we have found useful is to calculate

$$p_j(\infty) = d_j/D, \quad j = 1, 2, \dots, k, \tag{1.25}$$

where

$$D = d_1+d_2+\dots+d_k,$$

and d_j represents the determinant of the matrix found by deleting the jth row and jth column of $\boldsymbol{Q}$ (i.e. the jth principal minor of order $k-1$ of $\boldsymbol{Q}$); this is the cofactor of q_{jj}. (We are grateful to the late H. Kestelman for proving 1.25.) We may also note that D is equal to the product of the $k-1$ non-zero eigenvalues of $\boldsymbol{Q}$. Alternatively $\boldsymbol{p}(\infty)$ can be found either as $\boldsymbol{r}_1$ (see 1.6, and Colquhoun & Hawkes 1977), or by normalizing (to unit sum) any row of the adjoint matrix adj $(\boldsymbol{Q})$, because $\boldsymbol{Q}$ is singular and $\boldsymbol{p}(\infty)\boldsymbol{Q} = \boldsymbol{0}$ at equilibrium.

Experimental difficulties in the measurement of correlation

There are two main problems in practice. Firstly it is usually not possible to be sure that recordings are being made from a patch that contains only one active channel. The effect on the correlation coefficient of recording from several channels has not been systematically investigated. However it was pointed out above that correlations arise essentially from the occurrence of several sojourns in the same open state(s) in a burst. Thus, as long as the overall opening rate is low, it seems likely that the correlation may not be greatly affected by the presence of more than one channel.

The second problem arises from the limited time resolution that can be attained in practice. What appears to be an 'opening' at low resolution may actually be a 'burst' when looked at with higher resolution (see, for example, Colquhoun & Sakmann 1985). The interpretation of correlations between the lengths of openings is different from that between the lengths of bursts, as will be shown. But even at the best attainable resolution 'openings' may still actually be partially resolved bursts (see, for example, Colquhoun & Sakmann 1985). In such cases the correlation between burst lengths may be assessed accurately, but the correlation between open times, and between the lengths of the first and nth opening in a burst, may be seriously affected. The expected correlation coefficient may, in such cases, be calculated by the methods of Hawkes & Colquhoun (1987).

Numerical examples

Some examples that illustrate the correlations described in §§2–4 will be given in §5.

2. Correlations between open times and between shut times

In this section the states in which the system can exist are divided only into open states (subset $\mathscr{A}$) and shut states (subset $\mathscr{F}$), as described above and exemplified in figure 1.

Correlations between the durations of openings

The probability density function (PDF) of the lifetime of an opening is

$$f(t) = \boldsymbol{\phi}_0 \, e^{\boldsymbol{Q}_{\mathscr{A}\mathscr{A}}t} \boldsymbol{Q}_{\mathscr{A}\mathscr{F}} \boldsymbol{u}_{\mathscr{F}}$$
$$= \boldsymbol{\phi}_0 \, e^{\boldsymbol{Q}_{\mathscr{A}\mathscr{A}}t}(-\boldsymbol{Q}_{\mathscr{A}\mathscr{A}})\boldsymbol{u}_{\mathscr{A}}, \tag{2.1}$$

where the initial row vector, $\boldsymbol{\phi}_0$, gives the equilibrium probability that the opening starts in each of the open states (see Colquhoun & Hawkes 1982), and $\boldsymbol{u}_{\mathscr{A}}$ is a $k_{\mathscr{A}} \times 1$

column vector of units. In order to derive the correlation between the length (t_0) of an opening and the length (t_n) of the nth subsequent opening ($n = 1, 2, \ldots$) we use the bivariate distribution of t_0, t_n. This can be written by using the principles described for univariate distributions by Colquhoun & Hawkes (1982). It is

$$\left.\begin{aligned} f(t_0, t_n) &= \boldsymbol{\phi}_o \, e^{\boldsymbol{Q}_{\mathscr{A}\mathscr{A}} t_0} \boldsymbol{Q}_{\mathscr{A}\mathscr{F}} (\boldsymbol{G}_{\mathscr{F}\mathscr{A}} \boldsymbol{G}_{\mathscr{A}\mathscr{F}})^{n-1} \boldsymbol{G}_{\mathscr{F}\mathscr{A}} \, e^{\boldsymbol{Q}_{\mathscr{A}\mathscr{A}} t_n} \boldsymbol{Q}_{\mathscr{A}\mathscr{F}} \boldsymbol{u}_{\mathscr{F}}, \\ &= \boldsymbol{\phi}_o \, e^{\boldsymbol{Q}_{\mathscr{A}\mathscr{A}} t_0} (-\boldsymbol{Q}_{\mathscr{A}\mathscr{A}}) \boldsymbol{X}^n_{\mathscr{A}\mathscr{A}} \, e^{\boldsymbol{Q}_{\mathscr{A}\mathscr{A}} t_n} (-\boldsymbol{Q}_{\mathscr{A}\mathscr{A}}) \boldsymbol{u}_{\mathscr{A}}, \quad n \geqslant 1, \end{aligned}\right\} \tag{2.2}$$

and its Laplace transform, from (1.2), is

$$f^*(s_0, s_n) = \boldsymbol{\phi}_o (s_0 \boldsymbol{I} - \boldsymbol{Q}_{\mathscr{A}\mathscr{A}})^{-1} (-\boldsymbol{Q}_{\mathscr{A}\mathscr{A}}) \boldsymbol{X}^n_{\mathscr{A}\mathscr{A}} (s_n \boldsymbol{I} - \boldsymbol{Q}_{\mathscr{A}\mathscr{A}})^{-1} (-\boldsymbol{Q}_{\mathscr{A}\mathscr{A}}) \boldsymbol{u}_{\mathscr{A}}. \tag{2.3}$$

The average value of the product $t_0 t_n$ is, from (1.21) and (1.22),

$$E(t_0 t_n) = \boldsymbol{\phi}_o (-\boldsymbol{Q}^{-1}_{\mathscr{A}\mathscr{A}}) \boldsymbol{X}^n_{\mathscr{A}\mathscr{A}} (-\boldsymbol{Q}^{-1}_{\mathscr{A}\mathscr{A}}) \boldsymbol{u}_{\mathscr{A}}. \tag{2.4}$$

The mean length of an opening is

$$\mu_0 = \mu_n = \boldsymbol{\phi}_o (-\boldsymbol{Q}^{-1}_{\mathscr{A}\mathscr{A}}) \boldsymbol{u}_{\mathscr{A}} \tag{2.5}$$

and, from (2.1), (1.21) and (1.23) we obtain,

$$E(t_0^2) = E(t_n^2) = 2\boldsymbol{\phi}_o \boldsymbol{Q}^{-2}_{\mathscr{A}\mathscr{A}} \boldsymbol{u}_{\mathscr{A}},$$

so the variance of the open time is

$$\sigma^2_{\text{open}} = \boldsymbol{\phi}_o (-\boldsymbol{Q}^{-1}_{\mathscr{A}\mathscr{A}}) (2\boldsymbol{I} - \boldsymbol{u}_{\mathscr{A}} \boldsymbol{\phi}_o) (-\boldsymbol{Q}^{-1}_{\mathscr{A}\mathscr{A}}) \boldsymbol{u}_{\mathscr{A}}. \tag{2.6}$$

The autocorrelation coefficient with lag n is thus, from (1.20),

$$\rho(n) = \frac{\boldsymbol{\phi}_o (-\boldsymbol{Q}^{-1}_{\mathscr{A}\mathscr{A}}) [\boldsymbol{X}^n_{\mathscr{A}\mathscr{A}} - \boldsymbol{u}_{\mathscr{A}} \boldsymbol{\phi}_o] (-\boldsymbol{Q}^{-1}_{\mathscr{A}\mathscr{A}}) \boldsymbol{u}_{\mathscr{A}}}{\boldsymbol{\phi}_o (-\boldsymbol{Q}^{-1}_{\mathscr{A}\mathscr{A}}) [2\boldsymbol{I} - \boldsymbol{u}_{\mathscr{A}} \boldsymbol{\phi}_o] (-\boldsymbol{Q}^{-1}_{\mathscr{A}\mathscr{A}}) \boldsymbol{u}_{\mathscr{A}}}, \quad n \geqslant 1. \tag{2.7}$$

The $k_{\mathscr{A}} \times k_{\mathscr{A}}$ matrix $\boldsymbol{u}_{\mathscr{A}} \boldsymbol{\phi}_o$ has each row equal to $\boldsymbol{\phi}_o$, and therefore row sums equal to unity. Now let λ_i denote the eigenvalues of the $k_{\mathscr{A}} \times k_{\mathscr{A}}$ matrix $\boldsymbol{X}_{\mathscr{A}\mathscr{A}}$. This matrix has rows that sum to unity (see 1.9); it therefore has one unit eigenvalue, $\lambda_1 = 1$ say, the other eigenvalues being less than unity. The spectral expansion, from (1.5), can therefore be written as

$$\boldsymbol{X}^n_{\mathscr{A}\mathscr{A}} = \boldsymbol{A}_1 + \boldsymbol{A}_2 \lambda_2^n + \boldsymbol{A}_3 \lambda_3^n + \ldots, \tag{2.8}$$

where the matrices $\boldsymbol{A}_m$ can be found from the eigenvectors of $\boldsymbol{X}_{\mathscr{A}\mathscr{A}}$ (see (1.6)). We may also note that after many shut$\rightleftharpoons$open transitions ($n \to \infty$) the initial state (in $\mathscr{A}$) becomes irrelevant and the probability of starting an opening in each $\mathscr{A}$ state tends towards the equilibrium value $\boldsymbol{\phi}_o$, so it follows from (2.8) that

$$\lim_{n \to \infty} (\boldsymbol{X}^n_{\mathscr{A}\mathscr{A}}) = \boldsymbol{A}_1 = \boldsymbol{u}_{\mathscr{A}} \boldsymbol{\phi}_o, \tag{2.9}$$

thus the term in the numerator of the correlation coefficient can be written as

$$[\boldsymbol{X}^n_{\mathscr{A}\mathscr{A}} - \boldsymbol{u}_{\mathscr{A}} \boldsymbol{\phi}_o] = \boldsymbol{A}_2 \lambda_2^n + \boldsymbol{A}_3 \lambda_3^n + \ldots = \sum_{m=2}^{C} \boldsymbol{A}_m \lambda_m^n. \tag{2.10}$$

The decay of the correlation coefficient with increasing lag, n, can therefore be written in scalar form as

$$\rho(n) = w_2 \lambda_2^n + w_3 \lambda_3^n + \dots, \tag{2.11}$$

where the scalar coefficients are given, from (2.7) and (2.10), by

$$w_m = \frac{\boldsymbol{\phi}_o(-\boldsymbol{Q}_{\mathscr{A}\mathscr{A}}^{-1})\boldsymbol{A}_m(-\boldsymbol{Q}_{\mathscr{A}\mathscr{A}}^{-1})\boldsymbol{u}_{\mathscr{A}}}{\boldsymbol{\phi}_o(-\boldsymbol{Q}_{\mathscr{A}\mathscr{A}}^{-1})(2\boldsymbol{I}-\boldsymbol{u}_{\mathscr{A}}\boldsymbol{\phi}_o)(-\boldsymbol{Q}_{\mathscr{A}\mathscr{A}}^{-1})\boldsymbol{u}_{\mathscr{A}}}. \tag{2.12}$$

The form in (2.11) provides an alternative proof that the correlation decays towards zero as a sum of geometrically decaying terms (as in 2.11), as first shown by Fredkin *et al.* (1985). The number of terms in the sum in (2.10) is one fewer than the number of non-zero eigenvalues, i.e. it is usually $R(\boldsymbol{X}_{\mathscr{A}\mathscr{A}})-1 = C^{\mathrm{v}}(\mathscr{A},\mathscr{F})-1$. The connectivity between open and shut states, $C^{\mathrm{v}}(\mathscr{A},\mathscr{F})$ defined in §1, is therefore at least one plus the number of geometric components that are observed in the decay of the correlation coefficient.

The conditions for zero correlation

When the $\mathscr{A}$–$\mathscr{F}$ connectivity is 1, so $\boldsymbol{X}_{\mathscr{A}\mathscr{A}}$ has a rank of unity, the correlation between open times will be zero (all eigenvalues except $\lambda_1 = 1$ will be zero so (2.10) will be zero). This will, of course, be the case if there is only one sort of open state ($k_{\mathscr{A}} = 1$) or only one shut state ($k_{\mathscr{F}} = 1$); in either case $R(\boldsymbol{Q}_{\mathscr{A}\mathscr{F}}) = R(\boldsymbol{Q}_{\mathscr{F}\mathscr{A}}) = 1$. In fact in this case successive open times will be not just uncorrelated but also completely independent. This follows from the fact that the bivariate distribution, (2.2), can be factorized into the product of two scalars, one of which depends only on t_0 and the other only on t_n. This is the case because when $R(\boldsymbol{X}_{\mathscr{A}\mathscr{A}}) = 1$ we have from (1.6), (2.8) and (2.9), because $\lambda_1 = 1$,

$$\boldsymbol{X}_{\mathscr{A}\mathscr{A}}^n = \boldsymbol{A}_1 = \boldsymbol{c}_1 \boldsymbol{r}_1 = \boldsymbol{u}_{\mathscr{A}} \boldsymbol{\phi}_o, \tag{2.13}$$

so (2.2) can be written in the form

$$\left.\begin{aligned} f(t_0, t_n) &= [\boldsymbol{\phi}_o \mathrm{e}^{\boldsymbol{Q}_{\mathscr{A}\mathscr{A}} t_0}(-\boldsymbol{Q}_{\mathscr{A}\mathscr{A}})\boldsymbol{u}_{\mathscr{A}}][\boldsymbol{\phi}_o \mathrm{e}^{\boldsymbol{Q}_{\mathscr{A}\mathscr{A}} t_n}(-\boldsymbol{Q}_{\mathscr{A}\mathscr{A}})\boldsymbol{u}_{\mathscr{A}}], \\ &= f(t_0) f(t_n). \end{aligned}\right\} \tag{2.14}$$

This is simply the product of the two simple univariate exponential PDFS given in (2.1).

The maximum correlation

Perfect correlations would not be expected for a random process. It is of interest to see how large the correlation could be. Consider, for example, the following simple scheme.

$$\begin{array}{l} \mathrm{F}_4\text{—}\mathrm{A}_1 \\ \;| \\ \mathrm{F}_3\text{—}\mathrm{A}_2. \end{array}$$

The maximum correlation may be expected if $3 \rightleftharpoons 4$ transitions are slow (the extreme case of this is when there are two different independent channels). In this case $X^n_{\mathscr{A}\mathscr{A}}$ will approach an identity matrix, so the correlation will die out slowly and the maximum correlation will approach

$$\left[1+\frac{\phi_1+\phi_2(\tau_2/\tau_1)^2}{\phi_1\phi_2(1-\tau_2/\tau_1)^2}\right]^{-1}, \tag{2.15}$$

where τ_1 and τ_2 are the mean lifetimes of the two open states, and ϕ_1, ϕ_2 (the elements of $\boldsymbol{\phi}_o$) are the probabilities that an opening starts by transition to state 1 or to state 2, respectively ($\phi_1+\phi_2 = 1$). There will be no correlation if $\tau_1 = \tau_2$, and the greatest correlation will arise if the open lifetimes are very different ($\tau_2/\tau_1 \to 0$ or ∞). In the latter case the correlation could approach $\frac{1}{3}$ when $\phi_1 = \phi_2 = 0.5$ (both sorts of opening equally frequent) or 0.5 when $\phi_1 = \tau_2/(\tau_1+\tau_2)$ (so short openings are much more frequent).

Correlations between durations of shut periods

The correlation coefficient can be calculated from the same expression as for open times (2.7), after interchange of $\mathscr{A}$ and $\mathscr{F}$ throughout, and hence substitution for $\boldsymbol{\phi}_o$ of the initial vector for shut periods (see 6.4), namely $\boldsymbol{\phi}_s = \boldsymbol{\phi}_o \boldsymbol{G}_{\mathscr{A}\mathscr{F}}$. Thus the variance of shut times (cf. (2.6)) is

$$\sigma^2_{\text{shut}} = \boldsymbol{\phi}_s(-\boldsymbol{Q}^{-1}_{\mathscr{F}\mathscr{F}})[2\boldsymbol{I}-\boldsymbol{u}_{\mathscr{F}}\boldsymbol{\phi}_s](-\boldsymbol{Q}^{-1}_{\mathscr{F}\mathscr{F}})\boldsymbol{u}_{\mathscr{F}}, \tag{2.16}$$

and $$\rho(n) = \boldsymbol{\phi}_s(-\boldsymbol{Q}^{-1}_{\mathscr{F}\mathscr{F}})[(\boldsymbol{G}_{\mathscr{F}\mathscr{A}}\boldsymbol{G}_{\mathscr{A}\mathscr{F}})^n-\boldsymbol{u}_{\mathscr{F}}\boldsymbol{\phi}_s](-\boldsymbol{Q}^{-1}_{\mathscr{F}\mathscr{F}})\boldsymbol{u}_{\mathscr{F}}/\sigma^2_{\text{shut}}, \quad n \geqslant 1, \tag{2.17}$$

This will have the same form as (2.11), because the rank of $\boldsymbol{G}_{\mathscr{F}\mathscr{A}}\boldsymbol{G}_{\mathscr{A}\mathscr{F}}$ will be the same as that of $\boldsymbol{X}_{\mathscr{A}\mathscr{A}} = \boldsymbol{G}_{\mathscr{A}\mathscr{F}}\boldsymbol{G}_{\mathscr{F}\mathscr{A}}$.

We may note here that when the $\mathscr{A}$–$\mathscr{F}$ connectivity is unity, so that $\boldsymbol{Q}_{\mathscr{A}\mathscr{F}}$, etc. have unit rank, we have

$$\boldsymbol{G}_{\mathscr{A}\mathscr{F}} = \boldsymbol{u}_{\mathscr{A}}\boldsymbol{\phi}_s, \quad \boldsymbol{G}_{\mathscr{F}\mathscr{A}} = \boldsymbol{u}_{\mathscr{F}}\boldsymbol{\phi}_o, \tag{2.18}$$

which together imply the result in (2.13), and also that

$$\boldsymbol{G}_{\mathscr{F}\mathscr{A}}\boldsymbol{G}_{\mathscr{A}\mathscr{F}} = \boldsymbol{u}_{\mathscr{F}}\boldsymbol{\phi}_s, \tag{2.19}$$

so the correlation in (2.17) will be zero.

Thus correlations between shut times provide, in principle, similar information about the open–shut connectivity to that provided by correlations between open times (see examples in §5).

Correlation between open and shut times

The correlation coefficient between the length of an opening and the length of the nth subsequent shut period ($n \geqslant 1$) can be calculated by methods exactly analogous to those presented above. The result is

$$\rho(n) = \frac{\boldsymbol{\phi}_o(-\boldsymbol{Q}^{-1}_{\mathscr{A}\mathscr{A}})[\boldsymbol{X}^{n-1}_{\mathscr{A}\mathscr{A}}-\boldsymbol{u}_{\mathscr{A}}\boldsymbol{\phi}_o]\boldsymbol{G}_{\mathscr{A}\mathscr{F}}(-\boldsymbol{Q}^{-1}_{\mathscr{F}\mathscr{F}})\boldsymbol{u}_{\mathscr{F}}}{(\sigma^2_{\text{open}}\sigma^2_{\text{shut}})^{\frac{1}{2}}}, \tag{2.20}$$

where σ^2_{open} and σ^2_{shut} have been given in (2.6) and (2.16). As before, the correlation will be zero if the connectivity between the open states and the shut states is unity. An example is given in §5.

3. Correlations within bursts

When openings occur in bursts it may be possible (resolution permitting) to measure, for example, the correlation coefficient between the length of the first and of the nth opening in a burst ($n \geqslant 2$). For consistency with the notation elsewhere the openings in a burst should be numbered $0, 1, \ldots, n-1$, but the numbering $1, 2, \ldots, n$ will be retained here because of its familiarity. The lag of the autocorrelation coefficient is therefore $n-1$ rather than n, so it will be denoted $\rho(n-1)$. Thus $n = 2, 3, \ldots$ correspond to $\rho(1), \rho(2), \ldots$. These correlations should be independent of the number of channels in the patch from which measurements are made. Two versions of this procedure will be described. In the first version $\rho(n-1)$ is measured using all bursts that have n or more openings. In the second version $\rho(n-1)$ is measured using only those bursts that contain a fixed number of openings. There will be more data for the former calculation but the latter may give higher correlations.

Correlation between the first and the nth opening in all bursts with n or more openings

For any given n the data will consist of those bursts that have n or more openings ($n \geqslant 2$). The PDF of the length of the first opening in such bursts can be found by the methods of Colquhoun & Hawkes (1982) as

$$\begin{aligned} f(t_1) &= \boldsymbol{\phi}_\mathrm{b}\, \mathrm{e}^{\boldsymbol{Q}_{\mathscr{A}\mathscr{A}} t_1} \boldsymbol{Q}_{\mathscr{A}\mathscr{B}} \boldsymbol{G}_{\mathscr{B}\mathscr{A}} \boldsymbol{H}_{\mathscr{A}\mathscr{A}}^{n-2} \boldsymbol{u}_{\mathscr{A}} / P(r \geqslant n) \\ &= \boldsymbol{\phi}_\mathrm{b}\, \mathrm{e}^{\boldsymbol{Q}_{\mathscr{A}\mathscr{A}} t_1} (-\boldsymbol{Q}_{\mathscr{A}\mathscr{A}}) \boldsymbol{H}_{\mathscr{A}\mathscr{A}}^{n-1} \boldsymbol{u}_{\mathscr{A}} / P(r \geqslant n) \end{aligned} \tag{3.1}$$

and for the nth opening in such bursts the PDF is given by them as

$$f(t_n) = \boldsymbol{\phi}_\mathrm{b}\, \boldsymbol{H}_{\mathscr{A}\mathscr{A}}^{n-1} \mathrm{e}^{\boldsymbol{Q}_{\mathscr{A}\mathscr{A}} t_n} (-\boldsymbol{Q}_{\mathscr{A}\mathscr{A}}) \boldsymbol{u}_{\mathscr{A}} / P(r \geqslant n). \tag{3.2}$$

In these results the $1 \times k_{\mathscr{A}}$ vector $\boldsymbol{\phi}_\mathrm{b}$ contains the probabilities that the burst starts in each of the open states, and the denominator is the probability that a burst has n or more openings, namely

$$P(r \geqslant n) = \boldsymbol{\phi}_\mathrm{b}\, \boldsymbol{H}_{\mathscr{A}\mathscr{A}}^{n-1} \boldsymbol{u}_{\mathscr{A}}. \tag{3.3}$$

From these results we find the means as

$$\mu_1 = E(t_1) = \boldsymbol{\phi}_\mathrm{b} (-\boldsymbol{Q}_{\mathscr{A}\mathscr{A}}^{-1}) \boldsymbol{H}_{\mathscr{A}\mathscr{A}}^{n-1} \boldsymbol{u}_{\mathscr{A}} / P(r \geqslant n), \tag{3.4}$$

$$\mu_n = E(t_n) = \boldsymbol{\phi}_\mathrm{b}\, \boldsymbol{H}_{\mathscr{A}\mathscr{A}}^{n-1} (-\boldsymbol{Q}_{\mathscr{A}\mathscr{A}}^{-1}) \boldsymbol{u}_{\mathscr{A}} / P(r \geqslant n), \tag{3.5}$$

and, for calculation of the variances, we find that

$$E(t_1^2) = 2\boldsymbol{\phi}_\mathrm{b}\, \boldsymbol{Q}_{\mathscr{A}\mathscr{A}}^{-2} \boldsymbol{H}_{\mathscr{A}\mathscr{A}}^{n-1} \boldsymbol{u}_{\mathscr{A}} / P(r \geqslant n), \tag{3.6}$$

$$E(t_n^2) = 2\boldsymbol{\phi}_\mathrm{b}\, \boldsymbol{H}_{\mathscr{A}\mathscr{A}}^{n-1} \boldsymbol{Q}_{\mathscr{A}\mathscr{A}}^{-2} \boldsymbol{u}_{\mathscr{A}} / P(r \geqslant n). \tag{3.7}$$

In order to find the covariance we note that the bivariate distribution of t_1, t_n is

$$\begin{aligned} f(t_1, t_n) &= \boldsymbol{\phi}_\mathrm{b}\, \mathrm{e}^{\boldsymbol{Q}_{\mathscr{A}\mathscr{A}} t_1} \boldsymbol{Q}_{\mathscr{A}\mathscr{B}} \boldsymbol{G}_{\mathscr{B}\mathscr{A}} \boldsymbol{H}_{\mathscr{A}\mathscr{A}}^{n-2} \mathrm{e}^{\boldsymbol{Q}_{\mathscr{A}\mathscr{A}} t_n} (-\boldsymbol{Q}_{\mathscr{A}\mathscr{A}}) \boldsymbol{u}_{\mathscr{A}} / P(r \geqslant n) \\ &= \boldsymbol{\phi}_\mathrm{b}\, \mathrm{e}^{\boldsymbol{Q}_{\mathscr{A}\mathscr{A}} t_1} (-\boldsymbol{Q}_{\mathscr{A}\mathscr{A}}) \boldsymbol{H}_{\mathscr{A}\mathscr{A}}^{n-1} \mathrm{e}^{\boldsymbol{Q}_{\mathscr{A}\mathscr{A}} t_n} (-\boldsymbol{Q}_{\mathscr{A}\mathscr{A}}) \boldsymbol{u}_{\mathscr{A}} / P(r \geqslant n) \end{aligned} \tag{3.8}$$

so

$$E(t_1 t_n) = \boldsymbol{\phi}_\mathrm{b}\, \boldsymbol{Q}_{\mathscr{A}\mathscr{A}}^{-1} \boldsymbol{H}_{\mathscr{A}\mathscr{A}}^{n-1} \boldsymbol{Q}_{\mathscr{A}\mathscr{A}}^{-1} \boldsymbol{u}_{\mathscr{A}} / P(r \geqslant n). \tag{3.9}$$

Substutition of (3.4)–(3.7) and (3.9) into (1.19) gives the required autocorrelation coefficient, $\rho(n-1)$. The decay towards zero of the correlation coefficient as the lag, $n-1$, increases will have a more complex form than in the case of the correlation between open times. However, the condition for zero correlation is simple.

The condition for zero correlation

It can be shown that the correlation coefficient will be zero if $\boldsymbol{H}_{\mathscr{A}\mathscr{A}}$ has unit rank, i.e. if the direct connectivity between $\mathscr{A}$ states and $\mathscr{B}$ states, $D^{\mathrm{v}}(\mathscr{A},\mathscr{B}) = 1$. When $R(\boldsymbol{H}_{\mathscr{A}\mathscr{A}}) = 1$ all the eigenvalues of $\boldsymbol{H}_{\mathscr{A}\mathscr{A}}$ will be zero except for one, λ_1 say. Thus in this case, from (1.5 and 1.6),

$$\boldsymbol{H}^{n-1}_{\mathscr{A}\mathscr{A}} = \boldsymbol{A}_1\lambda_1^{n-1} = \boldsymbol{c}_1\boldsymbol{r}_1\lambda_1^{n-1}. \tag{3.10}$$

The expression for the autocorrelation coefficient shows that when

$$\boldsymbol{A}_1 = \boldsymbol{A}_1\boldsymbol{u}_{\mathscr{A}}\boldsymbol{\phi}_{\mathrm{b}}\boldsymbol{A}_1/\boldsymbol{\phi}_{\mathrm{b}}\boldsymbol{A}_1\boldsymbol{u}_{\mathscr{A}} \tag{3.11}$$

the correlation coefficient will be zero. It can be shown that this expression will be true whenever the direct connectivity between $\mathscr{A}$ and $\mathscr{B}$ is unity. The proof is particularly simple (via (1.6)) in the case where there is no direct communication between $\mathscr{A}$ and $\mathscr{C}$ because in this case $\boldsymbol{A}_1 = \boldsymbol{u}_{\mathscr{A}}\boldsymbol{\phi}_{\mathrm{b}}$ (also $\boldsymbol{\phi}_{\mathrm{b}} = \boldsymbol{\phi}_{\mathrm{o}}$ in this case).

The open times will not only be uncorrelated but also completely independent when $R(\boldsymbol{H}_{\mathscr{A}\mathscr{A}}) = 1$. As before (see (2.12), (2.14)) this follows from (1.6) or (3.10) which show that the bivariate distribution (3.8) factorizes into the product of two scalars. In fact, insertion of (3.11) into (3.8) shows that the bivariate distribution becomes the product of the univariate distributions in (3.1) and (3.2), i.e.

$$f(t_1, t_n) = f(t_1)f(t_n). \tag{3.12}$$

Correlation between the first and the nth opening in bursts with exactly r openings

This correlation, for lag $n-1$, will be denoted $\rho(n-1;r)$. The data consists of only those bursts which have exactly r openings. The distributions of the lengths of the first and the nth openings in such bursts are given by Colquhoun & Hawkes (1982) as

$$f(t_1) = \boldsymbol{\phi}_{\mathrm{b}}\,\mathrm{e}^{\boldsymbol{Q}_{\mathscr{A}\mathscr{A}}t_1}(-\boldsymbol{Q}_{\mathscr{A}\mathscr{A}})\,\boldsymbol{H}^{r-1}_{\mathscr{A}\mathscr{A}}\,\boldsymbol{e}_{\mathrm{b}}/P(r), \tag{3.13}$$

$$f(t_n) = \boldsymbol{\phi}_{\mathrm{b}}\,\boldsymbol{H}^{n-1}_{\mathscr{A}\mathscr{A}}\,\mathrm{e}^{\boldsymbol{Q}_{\mathscr{A}\mathscr{A}}t_n}(-\boldsymbol{Q}_{\mathscr{A}\mathscr{A}})\,\boldsymbol{H}^{r-n}_{\mathscr{A}\mathscr{A}}\,\boldsymbol{e}_{\mathrm{b}}/P(r), \quad r \geqslant n \geqslant 2, \tag{3.14}$$

with means

$$\mu_1 = \boldsymbol{\phi}_{\mathrm{b}}(-\boldsymbol{Q}^{-1}_{\mathscr{A}\mathscr{A}})\,\boldsymbol{H}^{r-1}_{\mathscr{A}\mathscr{A}}\,\boldsymbol{e}_{\mathrm{b}}/P(r), \tag{3.15}$$

$$\mu_n = \boldsymbol{\phi}_{\mathrm{b}}\,\boldsymbol{H}^{n-1}_{\mathscr{A}\mathscr{A}}(-\boldsymbol{Q}^{-1}_{\mathscr{A}\mathscr{A}})\,\boldsymbol{H}^{r-n}_{\mathscr{A}\mathscr{A}}\,\boldsymbol{e}_{\mathrm{b}}/P(r). \tag{3.16}$$

In these expressions the 'end of burst' vector is

$$\boldsymbol{e}_{\mathrm{b}} = (\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{C}} + \boldsymbol{G}_{\mathscr{A}\mathscr{C}})\,\boldsymbol{u}_{\mathscr{C}} = (\boldsymbol{I} - \boldsymbol{H}_{\mathscr{A}\mathscr{A}})\,\boldsymbol{u}_{\mathscr{A}} \tag{3.17}$$

and the probability of there being exactly r openings in a burst is

$$P(r) = \boldsymbol{\phi}_{\mathrm{b}}\,\boldsymbol{H}^{r-1}_{\mathscr{A}\mathscr{A}}\,\boldsymbol{e}_{\mathrm{b}}. \tag{3.18}$$

From (3.13) and (3.14) we also obtain, for calculation of the variance,

$$E(t_1^2) = 2\boldsymbol{\phi}_{\mathrm{b}}\, \boldsymbol{Q}_{\mathscr{A}\mathscr{A}}^{-2}\, \boldsymbol{H}_{\mathscr{A}\mathscr{A}}^{r-1}\, \boldsymbol{e}_{\mathrm{b}}/P(r), \tag{3.19}$$

$$E(t_n^2) = 2\boldsymbol{\phi}_{\mathrm{b}}\, \boldsymbol{H}_{\mathscr{A}\mathscr{A}}^{n-1}\, \boldsymbol{Q}_{\mathscr{A}\mathscr{A}}^{-2}\, \boldsymbol{H}_{\mathscr{A}\mathscr{A}}^{r-n}\, \boldsymbol{e}_{\mathrm{b}}/P(r). \tag{3.20}$$

The bivariate distribution of the open times is

$$f(t_1, t_n) = \boldsymbol{\phi}_{\mathrm{b}}\, \mathrm{e}^{\boldsymbol{Q}_{\mathscr{A}\mathscr{A}} t_1}(-\boldsymbol{Q}_{\mathscr{A}\mathscr{A}})\, \boldsymbol{H}_{\mathscr{A}\mathscr{A}}^{n-1}\, \mathrm{e}^{\boldsymbol{Q}_{\mathscr{A}\mathscr{A}} t_n}(-\boldsymbol{Q}_{\mathscr{A}\mathscr{A}})\, \boldsymbol{H}_{\mathscr{A}\mathscr{A}}^{r-n}\, \boldsymbol{e}_{\mathrm{b}}/P(r), \quad r \geqslant n \geqslant 2, \tag{3.21}$$

and therefore

$$E(t_1 t_n) = \boldsymbol{\phi}_{\mathrm{b}}(-\boldsymbol{Q}_{\mathscr{A}\mathscr{A}}^{-1})\, \boldsymbol{H}_{\mathscr{A}\mathscr{A}}^{n-1}(-\boldsymbol{Q}_{\mathscr{A}\mathscr{A}}^{-1})\, \boldsymbol{H}_{\mathscr{A}\mathscr{A}}^{r-n}\, \boldsymbol{e}_{\mathrm{b}}/P(r). \tag{3.22}$$

Substitution of these results into (1.19) gives the required correlation coefficients, $\rho(n-1; r)$.

As in the previous case, the correlation will be zero when (3.11) is true, which will be the case when $R(\boldsymbol{H}_{\mathscr{A}\mathscr{A}}) = 1$. Again openings are independent, as in (3.12), in this case. When the direct connectivity between $\mathscr{A}$ and $\mathscr{B}$ is greater than one there will be a correlation which will decay towards zero with increasing lag $(n-1)$.

Examples of burst mechanisms

Figure 2 shows various possible arrangements of the states. The rank of $\boldsymbol{Q}_{\mathscr{A}\mathscr{B}}$ (and hence, usually, that of $\boldsymbol{H}_{\mathscr{A}\mathscr{A}}$) can be determined by inspection in the way described in §1 for the rank of $\boldsymbol{Q}_{\mathscr{A}\mathscr{F}}$. In all the reaction schemes shown in figure 2, except for **6**, **15** and **19–23**, the direct $\mathscr{A}$–$\mathscr{B}$ connectivity is $D^{\mathrm{v}}(\mathscr{A}, \mathscr{B}) = 1$ so $R(\boldsymbol{H}_{\mathscr{A}\mathscr{A}}) = 1$ and no correlations within bursts would be found. In the remainder $R(\boldsymbol{H}_{\mathscr{A}\mathscr{A}}) = 2$ so such correlations would be present. A numerical example is given in §5.

4. Correlation between bursts

Two sorts of correlation will be considered, the correlations between the lengths of successive bursts, and correlations between the numbers of openings in successive bursts. Both sorts give similar information in principle.

Correlations between the lengths of bursts

We wish to calculate the correlation between the length, t_0, of a burst and the length, t_n, of the nth subsequent burst $(n = 1, 2, \ldots)$. The Laplace transform of the PDF for the length of a burst is given by Colquhoun & Hawkes (1982) as

$$f^*(s) = \boldsymbol{\phi}_{\mathrm{b}}[\boldsymbol{I} - \boldsymbol{G}_{\mathscr{A}\mathscr{B}}^*(s)\, \boldsymbol{G}_{\mathscr{B}\mathscr{A}}^*(s)]^{-1}[\boldsymbol{G}_{\mathscr{A}\mathscr{B}}^*(s)\, \boldsymbol{G}_{\mathscr{B}\mathscr{C}} + \boldsymbol{G}_{\mathscr{A}\mathscr{C}}^*(s)]\, \boldsymbol{u}_{\mathscr{C}} \tag{4.1}$$

with mean, for any burst,

$$\mu_0 = \mu_n = \boldsymbol{\phi}_{\mathrm{b}}\, \boldsymbol{Y}_{\mathscr{A}\mathscr{A}}\, \boldsymbol{u}_{\mathscr{A}}, \tag{4.2}$$

where we define, for brevity,

$$\boldsymbol{Y}_{\mathscr{A}\mathscr{A}} = (\boldsymbol{I} - \boldsymbol{G}_{\mathscr{A}\mathscr{B}}\, \boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{-1}(-\boldsymbol{Q}_{\mathscr{A}\mathscr{A}}^{-1})(\boldsymbol{I} - \boldsymbol{Q}_{\mathscr{A}\mathscr{B}}\, \boldsymbol{Q}_{\mathscr{B}\mathscr{B}}^{-1}\, \boldsymbol{G}_{\mathscr{B}\mathscr{A}}). \tag{4.3}$$

From (4.1) and (1.21) we find also, for calculation of the variance,

$$E(t_0^2) = E(t_n^2) = \mathrm{d}^2 f^*(s)/\mathrm{d}s^2|_{s=0} = 2\boldsymbol{\phi}_{\mathrm{b}}[\boldsymbol{Y}_{\mathscr{A}\mathscr{A}}^2 + (\boldsymbol{I} - \boldsymbol{H}_{\mathscr{A}\mathscr{A}})^{-1}\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\, \boldsymbol{Q}_{\mathscr{B}\mathscr{B}}^{-2}\, \boldsymbol{G}_{\mathscr{B}\mathscr{A}}]\, \boldsymbol{u}_{\mathscr{A}} \tag{4.4}$$

To calculate $E(t_0 t_n)$ we require the Laplace transform of the bivariate distribution of t_0, t_n, which is

$$f^*(s_0, s_n) = \boldsymbol{\phi}_{\mathrm{b}}[\boldsymbol{I}-\boldsymbol{G}^*_{\mathscr{A}\mathscr{B}}(s_0)\,\boldsymbol{G}^*_{\mathscr{B}\mathscr{A}}(s_0)]^{-1}[\boldsymbol{G}^*_{\mathscr{A}\mathscr{B}}(s_0)\,\boldsymbol{G}_{\mathscr{B}\mathscr{C}}+\boldsymbol{G}^*_{\mathscr{A}\mathscr{C}}(s_0)]\,(\boldsymbol{I}-\boldsymbol{G}_{\mathscr{C}\mathscr{B}}\,\boldsymbol{G}_{\mathscr{B}\mathscr{C}})^{-1}$$

$$(\boldsymbol{G}_{\mathscr{C}\mathscr{B}}\,\boldsymbol{G}_{\mathscr{B}\mathscr{A}}+\boldsymbol{G}_{\mathscr{C}\mathscr{A}})\,\boldsymbol{Z}^{n-1}_{\mathscr{A}\mathscr{A}}[\boldsymbol{I}-\boldsymbol{G}^*_{\mathscr{A}\mathscr{B}}(s_n)\,\boldsymbol{G}^*_{\mathscr{B}\mathscr{A}}(s_n)]^{-1}[\boldsymbol{G}^*_{\mathscr{A}\mathscr{B}}(s_n)\,\boldsymbol{G}_{\mathscr{B}\mathscr{C}}+\boldsymbol{G}^*_{\mathscr{A}\mathscr{C}}(s_n)]\,\boldsymbol{u}_{\mathscr{C}}, \quad (4.5)$$

where $\boldsymbol{Z}_{\mathscr{A}\mathscr{A}}$, the $k_{\mathscr{A}} \times k_{\mathscr{A}}$ probability transition matrix for passage from the start of one burst to the start of the next (Colquhoun & Hawkes 1982), has been defined in (1.11)–(1.13).

It may be noted that $\boldsymbol{Z}_{\mathscr{A}\mathscr{A}}$ is analogous with $\boldsymbol{X}_{\mathscr{A}\mathscr{A}} = \boldsymbol{G}_{\mathscr{A}\mathscr{F}}\,\boldsymbol{G}_{\mathscr{F}\mathscr{A}}$ in §2, which is the probability transition matrix from the start of one opening to the start of the next.

Thus, from (1.21) and (4.5)

$$E(t_0 t_n) = \partial f^*(s_0, s_n)/\partial s_0\,\partial s_n|_{s=0} = \boldsymbol{\phi}_{\mathrm{b}}\,\boldsymbol{Y}_{\mathscr{A}\mathscr{A}}\,\boldsymbol{Z}^n_{\mathscr{A}\mathscr{A}}\,\boldsymbol{Y}_{\mathscr{A}\mathscr{A}}\,\boldsymbol{u}_{\mathscr{A}}. \quad (4.6)$$

Substitution of (4.2), (4.4) and (4.6) into (1.20) gives the correlation coefficient as

$$\rho(n) = \boldsymbol{\phi}_{\mathrm{b}}\,\boldsymbol{Y}_{\mathscr{A}\mathscr{A}}(\boldsymbol{Z}^n_{\mathscr{A}\mathscr{A}}-\boldsymbol{u}_{\mathscr{A}}\,\boldsymbol{\phi}_{\mathrm{b}})\,\boldsymbol{Y}_{\mathscr{A}\mathscr{A}}\,\boldsymbol{u}_{\mathscr{A}}/\sigma^2, \quad n \geqslant 1, \quad (4.7)$$

where

$$\sigma^2 = \boldsymbol{\phi}_{\mathrm{b}}\,\boldsymbol{Y}_{\mathscr{A}\mathscr{A}}(2\boldsymbol{I}-\boldsymbol{u}_{\mathscr{A}}\,\boldsymbol{\phi}_{\mathrm{b}})\,\boldsymbol{Y}_{\mathscr{A}\mathscr{A}}\,\boldsymbol{u}_{\mathscr{A}}+2\boldsymbol{\phi}_{\mathrm{b}}(\boldsymbol{I}-\boldsymbol{H}_{\mathscr{A}\mathscr{A}})^{-1}\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\,\boldsymbol{Q}^{-2}_{\mathscr{B}\mathscr{B}}\,\boldsymbol{G}_{\mathscr{B}\mathscr{A}}\,\boldsymbol{u}_{\mathscr{A}}. \quad (4.8)$$

The characteristics of the correlation coefficient (4.7) are rather like those of correlation between open times (2.7). The burst transition matrix has rows that sum to unity (see (1.14)), as has $\boldsymbol{X}_{\mathscr{A}\mathscr{A}}$. Therefore $\boldsymbol{Z}_{\mathscr{A}\mathscr{A}}$ has one unit eigenvalue, $\lambda_1 = 1$ say, the other eigenvalues being less than unity. The spectral expansion, from (1.5), can be written as

$$\boldsymbol{Z}^n_{\mathscr{A}\mathscr{A}} = \boldsymbol{A}_1+\boldsymbol{A}_2\,\lambda_2^n+\boldsymbol{A}_3\,\lambda_3^n+\ldots, \quad (4.9)$$

where the matrices $\boldsymbol{A}_m$ can be found from the eigenvectors of $\boldsymbol{Z}_{\mathscr{A}\mathscr{A}}$ (see 1.6). After many transitions the initial state (in $\mathscr{A}$) becomes irrelevant so we find that

$$\lim_{n\to\infty} (\boldsymbol{Z}^n_{\mathscr{A}\mathscr{A}}) = \boldsymbol{A}_1 = \boldsymbol{u}_{\mathscr{A}}\,\boldsymbol{\phi}_{\mathrm{b}} \quad (4.10)$$

a result analogous with that in (2.9) for open times.

The central term in the numerator of the correlation coefficient can, from (4.9), be written as

$$\boldsymbol{Z}^n_{\mathscr{A}\mathscr{A}}-\boldsymbol{u}_{\mathscr{A}}\,\boldsymbol{\phi}_{\mathrm{b}} = \boldsymbol{A}_2\,\lambda_2^n+\boldsymbol{A}_3\,\lambda_3^n+\ldots, \quad (4.11)$$

therefore the correlation coefficient will be zero if $\boldsymbol{Z}_{\mathscr{A}\mathscr{A}}$ has unit rank (so $\lambda_2 = \lambda_3 = \ldots = 0$). In fact burst lengths will be completely independent in this case as was found for open times. Otherwise the correlation will decay towards zero, with increasing lag (n), as the sum of $R(\boldsymbol{Z}_{\mathscr{A}\mathscr{A}})-1$ geometrically decaying terms. This result is exactly analogous with that for open times (see (2.7)–(2.12)); thus observations on the correlations between burst lengths can, in principle, give information about the rank of $\boldsymbol{Z}_{\mathscr{A}\mathscr{A}}$, just as observations on the correlation between open times can give information about the rank of $\boldsymbol{X}_{\mathscr{A}\mathscr{A}}$. The interpretation of such observations is discussed below.

Correlations between numbers of openings in bursts

The correlation coefficient between the number (r_0) of openings in a burst and the number (r_n) in the nth subsequent burst $(n = 1, 2, \ldots)$ can be calculated as follows.

$$P(r) = \boldsymbol{\phi}_{\mathrm{b}} \boldsymbol{H}_{\mathscr{A}\mathscr{A}}^{r-1}(\boldsymbol{I}-\boldsymbol{H}_{\mathscr{A}\mathscr{A}})\,\boldsymbol{u}_{\mathscr{A}}, \tag{4.12}$$

$$\mu_0 = \mu_n = \boldsymbol{\phi}_{\mathrm{b}}(\boldsymbol{I}-\boldsymbol{H}_{\mathscr{A}\mathscr{A}})^{-1}\boldsymbol{u}_{\mathscr{A}}, \tag{4.13}$$

$$E(r_0^2) = E(r_n^2) = \boldsymbol{\phi}_{\mathrm{b}}(\boldsymbol{I}+\boldsymbol{H}_{\mathscr{A}\mathscr{A}})(\boldsymbol{I}-\boldsymbol{H}_{\mathscr{A}\mathscr{A}})^{-2}\boldsymbol{u}_{\mathscr{A}} \tag{4.14}$$

so

$$\operatorname{var}(r) = \boldsymbol{\phi}_{\mathrm{b}}(\boldsymbol{I}-\boldsymbol{H}_{\mathscr{A}\mathscr{A}})^{-1}(\boldsymbol{I}+\boldsymbol{H}_{\mathscr{A}\mathscr{A}}-\boldsymbol{u}_{\mathscr{A}}\boldsymbol{\phi}_{\mathrm{b}})(\boldsymbol{I}-\boldsymbol{H}_{\mathscr{A}\mathscr{A}})^{-1}\boldsymbol{u}_{\mathscr{A}}. \tag{4.15}$$

The bivariate distribution of r_0, r_n is

$$P(r_0, r_n) = \boldsymbol{\phi}_{\mathrm{b}} \boldsymbol{H}_{\mathscr{A}\mathscr{A}}^{r_0-1}(\boldsymbol{I}-\boldsymbol{H}_{\mathscr{A}\mathscr{A}})\,\boldsymbol{Z}_{\mathscr{A}\mathscr{A}}^{n}\,\boldsymbol{H}_{\mathscr{A}\mathscr{A}}^{r_n-1}(\boldsymbol{I}-\boldsymbol{H}_{\mathscr{A}\mathscr{A}})\,\boldsymbol{u}_{\mathscr{A}}, \tag{4.16}$$

so

$$E(r_0 r_n) = \boldsymbol{\phi}_{\mathrm{b}}(\boldsymbol{I}-\boldsymbol{H}_{\mathscr{A}\mathscr{A}})^{-1}\boldsymbol{Z}_{\mathscr{A}\mathscr{A}}^{n}(\boldsymbol{I}-\boldsymbol{H}_{\mathscr{A}\mathscr{A}})^{-1}\boldsymbol{u}_{\mathscr{A}}. \tag{4.17}$$

The correlation coefficient is therefore

$$\rho(n) = \boldsymbol{\phi}_{\mathrm{b}}(\boldsymbol{I}-\boldsymbol{H}_{\mathscr{A}\mathscr{A}})^{-1}(\boldsymbol{Z}_{\mathscr{A}\mathscr{A}}^{n}-\boldsymbol{u}_{\mathscr{A}}\boldsymbol{\phi}_{\mathrm{b}})(\boldsymbol{I}-\boldsymbol{H}_{\mathscr{A}\mathscr{A}})^{-1}\boldsymbol{u}_{\mathscr{A}}/\operatorname{var}(r), \quad n \geqslant 1. \tag{4.18}$$

The central part of this equation is the same as that for the correlation of burst lengths, so similar information about the rank of $\boldsymbol{Z}_{\mathscr{A}\mathscr{A}}$ can be inferred; there will be no correlation if $R(\boldsymbol{Z}_{\mathscr{A}\mathscr{A}}) = 1$.

Connections between states and the rank of $\boldsymbol{Z}_{\mathscr{A}\mathscr{A}}$

As in previous cases, it is possible, by inspection of the reaction scheme, to see what the rank of the appropriate matrix ($\boldsymbol{Z}_{\mathscr{A}\mathscr{A}}$ in this case) will be, and hence whether or not correlations between bursts would be expected. It was pointed out in §1 that the rank of $\boldsymbol{Z}_{\mathscr{A}\mathscr{A}}$ will usually be the same as the $\mathscr{A}$–$\mathscr{C}$ connectivity, $C^{\mathrm{v}}(\mathscr{A}, \mathscr{C})$. As expected from the definitions of $\boldsymbol{Z}_{\mathscr{A}\mathscr{A}}$ in (1.13), the same rank will usually be shared also by the matrices $\boldsymbol{G}_{\mathscr{A}(\mathscr{B})\mathscr{C}}$, $\boldsymbol{G}_{\mathscr{C}(\mathscr{B})\mathscr{A}}$, $(\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{C}}+\boldsymbol{G}_{\mathscr{A}\mathscr{C}})$ and $(\boldsymbol{G}_{\mathscr{C}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}}+\boldsymbol{G}_{\mathscr{C}\mathscr{A}})$. These describe the routes from the start of a burst (in $\mathscr{A}$) to its end (in $\mathscr{C}$), possibly via $\mathscr{B}$, and back again to $\mathscr{A}$.

In some cases it will be obvious that no correlation is expected, i.e. that $R(\boldsymbol{Z}_{\mathscr{A}\mathscr{A}}) = 1$. It is clear from the definition of $\boldsymbol{Z}_{\mathscr{A}\mathscr{A}}$ in (1.13) that $R(\boldsymbol{Z}_{\mathscr{A}\mathscr{A}})$ depends on the rank of a $k_{\mathscr{A}} \times k_{\mathscr{C}}$ matrix so its rank cannot exceed $\min(k_{\mathscr{A}}, k_{\mathscr{C}})$. Therefore if there is only one open state, or only one $\mathscr{C}$ state, the rank of $\boldsymbol{Z}_{\mathscr{A}\mathscr{A}}$, and the $\mathscr{A}$–$\mathscr{C}$ connectivity, must be 1 and no correlations between bursts will be expected (as, for example, in figure 2, **1–6**). From (1.11)–(1.13), (1.17) and (1.18) it follows that the rank of $\boldsymbol{Z}_{\mathscr{A}\mathscr{A}}$ and the $\mathscr{A}$–$\mathscr{C}$ connectivity will be 1, and so no correlations between bursts will be expected if one (or more) of the following conditions is true: (*a*) if the direct $\mathscr{A}$–$\mathscr{B}$ connectivity is 1, so $R(\boldsymbol{Q}_{\mathscr{A}\mathscr{B}}) = 1$, *and* there are no direct routes from $\mathscr{A}$ states to $\mathscr{C}$ states, so $R(\boldsymbol{Q}_{\mathscr{A}\mathscr{C}}) = 0$ (e.g. figure 2, **8** and **12**); (*b*) if $R(\boldsymbol{Q}_{\mathscr{B}\mathscr{C}}) = 1$ *and* $R(\boldsymbol{Q}_{\mathscr{A}\mathscr{C}}) = 0$ (e.g. figure 2, **15**); (*c*) if $R(\boldsymbol{Q}_{\mathscr{A}\mathscr{C}}) = 1$ *and* $R(\boldsymbol{Q}_{\mathscr{B}\mathscr{C}}) = 0$ (e.g. figure 2, **10**, **11**, **13**, **14** and **21**); (*d*) if, from the third definition in (1.13), connectivity is 1 between the burst states ($\mathscr{E} = \mathscr{A} \cup \mathscr{B}$) and the $\mathscr{C}$ states so $R(\boldsymbol{Q}_{\mathscr{E}\mathscr{C}}) = 1$ (e.g. figure 2, **21**), or between shut states and open states so $R(\boldsymbol{Q}_{\mathscr{F}\mathscr{A}}) = 1$.

In most of the cases in figure 2 in which $R(\boldsymbol{Z}_{\mathscr{A}\mathscr{A}}) = 1$, one or more of the conditions listed above is true. However, for **9** and **20**, $C^{\mathrm{v}}(\mathscr{A},\mathscr{C})=1$ and $R(\boldsymbol{Z}_{\mathscr{A}\mathscr{A}})=1$ though none of the above conditions holds. In **9** deletion of the gateway state (A_1) completely disconnects $\mathscr{A}$ and $\mathscr{C}$. For **20** $R(\boldsymbol{Q}_{\mathscr{A}\mathscr{C}})=0$, but $R(\boldsymbol{X}_{\mathscr{A}\mathscr{A}})=R(\boldsymbol{H}_{\mathscr{A}\mathscr{A}})=R(\boldsymbol{Q}_{\mathscr{A}\mathscr{B}})=R(\boldsymbol{Q}_{\mathscr{B}\mathscr{C}})=R(\boldsymbol{Q}_{\mathscr{E}\mathscr{C}})=R(\boldsymbol{Q}_{\mathscr{F}\mathscr{A}})=2$. Nevertheless, $R(\boldsymbol{Z}_{\mathscr{A}\mathscr{A}})=1$ and the $\mathscr{A}$–$\mathscr{C}$ connectivity, $C^{\mathrm{v}}(\mathscr{A},\mathscr{C})=1$ because deletion of state B_4 disconnects $\mathscr{A}$ and $\mathscr{C}$ completely. So $R(\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{C}})=1$, i.e. the equality sign in (1.17) does not hold; and although $\boldsymbol{G}_{\mathscr{E}\mathscr{C}}$ and $\boldsymbol{G}_{\mathscr{F}\mathscr{A}}$ both have rank 2, their subsections that appear in (1.13), $(\boldsymbol{G}_{\mathscr{E}\mathscr{C}})_{\mathscr{A}\mathscr{C}}$ and $(\boldsymbol{G}_{\mathscr{F}\mathscr{A}})_{\mathscr{C}\mathscr{A}}$, both have rank 1. Clearly the unit $\mathscr{A}$–$\mathscr{C}$ connectivity, and the lack of correlations between bursts, in this example results from the fact that during passage from $\mathscr{A}$ states to $\mathscr{C}$ states (and vice versa) the system must always pass through one single state (the $\mathscr{B}$ state numbered 4 in figure 2, **20**); what happens after leaving state 4 must be independent of what happened earlier.

5. Numerical examples of correlations

Consider the following scheme, with rate constants in reciprocal seconds as shown on the arrows:

$$\begin{array}{ccc} \mathrm{C}_5 & \underset{1}{\overset{1}{\rightleftharpoons}} & \mathrm{C}_6 \\ {\scriptstyle 10}\downarrow\uparrow{\scriptstyle 100} & & \\ \mathrm{B}_4 & \underset{10^4}{\overset{1000}{\rightleftharpoons}} & \mathrm{A}_1 \\ {\scriptstyle 20}\downarrow\uparrow{\scriptstyle 1000} & & \\ \mathrm{B}_3 & \underset{100}{\overset{10^4}{\rightleftharpoons}} & \mathrm{A}_2. \end{array} \tag{5.1}$$

This is like **15** in figure 2 (apart from connections *within* the open states, which do not affect the presence or absence of correlations). It is expected (see figure 2) to show correlations between open times and within bursts, but not between bursts. The correlation between open times should be relatively large because (see (2.15)), the $\mathrm{B}_3 \rightleftharpoons \mathrm{B}_4$ interchange is not very fast, the mean lifetime of A_1 (0.1 ms) is much shorter than that of A_2 (10 ms), but the former is more common because $\boldsymbol{\phi}_0=[0.83\ \ 0.17]$. The correlations die out rather slowly (see (2.11)), the non-unit eigenvalue, λ_2, of $\boldsymbol{X}_{\mathscr{A}\mathscr{A}}$ (and also of $\boldsymbol{G}_{\mathscr{F}\mathscr{A}}\boldsymbol{G}_{\mathscr{A}\mathscr{F}}$) being 0.893. The results are shown in table 1*a*.

A scheme with the same states, but different connections between them is shown (with values for the rate constants) in (5.2). This scheme (apart from connections *within* open states) is like **16** in figure 2.

$$\begin{array}{ccc} \mathrm{C}_5 & \underset{1}{\overset{1}{\rightleftharpoons}} & \mathrm{C}_6 \\ {\scriptstyle 10}\downarrow\uparrow{\scriptstyle 100} & & {\scriptstyle 10}\downarrow\uparrow{\scriptstyle 10^4} \\ \mathrm{B}_4 & & \mathrm{A}_1 \\ {\scriptstyle 20}\downarrow\uparrow{\scriptstyle 1000} & & \\ \mathrm{B}_3 & \underset{100}{\overset{10^4}{\rightleftharpoons}} & \mathrm{A}_2. \end{array} \tag{5.2}$$

TABLE 1. CORRELATION COEFFICIENTS AS A FUNCTION OF THE LAG, l.

(Results are shown for (*a*) the example given in (5.1), and (*b*) the example in (5.2). The open-time correlation is the correlation between the length of an opening and the length of the nth subsequent opening, from (2.11) and (2.12). The shut-time correlation, from (2.17) is similar. The open–shut correlation is that between the length of an opening and the nth subsequent shut time, from (2.20). The correlation between burst lengths is found from (4.7), and that between the number of openings per burst come from (4.18). In all of these cases the lag (column 1) is $l = n$.

For the correlations between openings within bursts, $\rho(l)$ gives the correlation between the length of the first opening in the burst and that of the nth opening in the same burst; and $\rho(l;r)$ is the same, but for bursts with exactly r openings only (tabulated up to $r = 5$); for both of these cases the lag for the autocorrelation coefficient is $l = n-1$ (see §3). For example $\rho(2;4) = 0.201$ is the correlation between the length of the first and third openings in bursts with exactly four openings.)

(*a*) *The example in* (5.1)

	open and shut times			openings within bursts		between bursts	
	open	shut	open–shut			burst length	openings per burst
lag (l)	$\rho\ (l)$	$\rho\ (l)$	$\rho\ (l)$	$\rho\ (l)$	$\rho\ (l;r)$	$\rho\ (l)$	$\rho\ (l)$
1	0.401	0.0020	−0.030	0.328	0.245 ($r = 2$) 0.284 ($r = 3$) 0.301 ($r = 4$) 0.311 ($r = 5$)	0	0
2	0.358	0.0018	−0.027	0.254	0.164 ($r = 3$) 0.201 ($r = 4$) 0.220 ($r = 5$)	0	0
3	0.320	0.0016	−0.024	0.209	0.124 ($r = 4$) 0.156 ($r = 5$)	0	0
4	0.286	0.0014	−0.022	0.177	0.099 ($r = 5$)	0	0
5	0.255	0.0013	−0.019	0.154	—	0	0
⋮							
∞	0	0	0	0	0	0	0

(*b*) *The example in* (5.2)

lag (l)	open $\rho\ (l)$	shut $\rho\ (l)$	open–shut $\rho\ (l)$	$\rho\ (l)$	$\rho\ (l;r)$	burst length $\rho\ (l)$	openings per burst $\rho\ (l)$
1	0.225	0.054	−0.115	0	0	0.265	0.254
2	0.206	0.049	−0.105	0	0	0.151	0.145
3	0.188	0.045	−0.096	0	0	0.086	0.083
4	0.172	0.041	−0.088	0	0	0.049	0.047
5	0.157	0.037	−0.080	0	0	0.028	0.027
⋮							
∞	0	0	0	0	0	0	0

This scheme will have correlations between open times and between bursts, but not within bursts (see figure 2).

The mean lifetimes of the open states are the same as in (5.1) though short openings are less common in this case, $\boldsymbol{\phi}_0 = [0.33 \;\; 0.67]$. The distribution of the number of openings per burst has components with means of 13 and 1 (see §7), the former consisting of 10 ms openings (A_2), and the latter of 0.1 ms openings. The unit mean component has 87 % of the area, cf. $\boldsymbol{\phi}_b = [0.87 \;\; 0.13]$. The predicted correlations are summarized in table 1*b*. Again the correlations between open and shut times die out slowly; the eigenvalues of $\boldsymbol{X}_{\mathscr{A}\mathscr{A}}$ are $\lambda_1 = 1$, $\lambda_2 = 0.914$ so there is a factor of 0.914 between successive correlation coefficients (see (2.11)).

Correlations between bursts are slightly larger, but die out faster; the eigenvalues of $\boldsymbol{Z}_{\mathscr{A}\mathscr{A}}$ are $\lambda_1 = 1$ and $\lambda_2 = 0.571$, so there is a factor of 0.571 between successive correlation coefficients (see (4.11)). If the interchange between the two $\mathscr{C}$ states were faster the correlations would be smaller.

6. Distributions of channel openings after a perturbation

Many sorts of experiment produce single-channel records that are not in a steady state. This will be the case, for example, when ion channels are observed after a sudden change in membrane potential (voltage jump) or a sudden change in agonist concentration (concentration jump). The relevant distributions follow directly from the approach used by Colquhoun & Hawkes (1982) as long as (*a*) there is only one channel present and (*b*) the perturbation is applied as a step (at time $t = 0$, say) so that at all times greater than zero the transition rates are constant. It may be noted that although the term *equilibrium* is used here, the results actually apply to systems that are maintained in a *steady state* by an energy supply (Colquhoun & Hawkes 1983).

For example, the distribution of the length of the first opening of a channel after a voltage jump may not be the same as the usual equilibrium distribution. It will be shown that the condition for such distributions to be the same as at equilibrium is simply that there is no correlation between open times, etc.

We may, however, note here that it is only the relative areas of the components of the distributions that are expected to differ from those observed at equilibrium. In every case the time constants should be the same as at equilibrium, except for the latencies to the first burst in (6.16) and (6.19), which will have the time constants of the distribution of all shut periods rather than those of the distribution of gaps between bursts.

We first consider the case where the states of the system are divided simply into open states ($\mathscr{A}$) and shut states ($\mathscr{F}$), and then the case where the openings occur in bursts.

Openings after a perturbation

The equilibrium distributions

At equilibrium the distribution of open times has a PDF

$$f(t) = \boldsymbol{\phi}_0 \, \mathrm{e}^{\boldsymbol{Q}_{\mathscr{A}\mathscr{A}} t} (-\boldsymbol{Q}_{\mathscr{A}\mathscr{A}}) \, \boldsymbol{u}_{\mathscr{A}}, \tag{6.1}$$

and the PDF for shut times (see (2.16))

$$f(t) = \boldsymbol{\phi}_0 \, \boldsymbol{G}_{\mathscr{A}\mathscr{F}} \, \mathrm{e}^{\boldsymbol{Q}_{\mathscr{F}\mathscr{F}} t} (-\boldsymbol{Q}_{\mathscr{F}\mathscr{F}}) \, \boldsymbol{u}_{\mathscr{F}}. \tag{6.2}$$

The equilibrium initial vector

The elements of the $(1 \times k_{\mathscr{A}})$ vector $\boldsymbol{\phi}_0$ give the equilibrium probabilities that an opening starts in each of the open states (it might, more appropriately, be denoted $\boldsymbol{\phi}_0(\infty)$ to emphasize its equilibrium nature). This may be calculated (Colquhoun & Hawkes 1982, equation (3.63)) as

$$\boldsymbol{\phi}_0 = \boldsymbol{p}_{\mathscr{F}}(\infty) \, \boldsymbol{Q}_{\mathscr{F}\mathscr{A}} / \boldsymbol{p}_{\mathscr{F}}(\infty) \, \boldsymbol{Q}_{\mathscr{F}\mathscr{A}} \, \boldsymbol{u}_{\mathscr{A}}, \tag{6.3}$$

where $\boldsymbol{p}_{\mathscr{F}}(\infty)$ gives the equilibrium occupancies of each of the shut states and $\boldsymbol{Q}_{\mathscr{F}\mathscr{A}}$ gives the transition rates from shut states to open states. This form is intuitively reasonable; the chance that an opening starts in a particular open state will tend to be large if either the shut state(s) that are connected to it are highly populated (occupancies in $\boldsymbol{p}_{\mathscr{F}}(\infty)$), or if the transition rates (in $\boldsymbol{Q}_{\mathscr{F}\mathscr{A}}$) from these shut states to the open state in question are rapid. The (scalar) constant in the denominator of (6.3) is present merely to ensure that the probabilities add to unity, i.e. $\boldsymbol{\phi}_{\mathrm{o}}\boldsymbol{u}_{\mathscr{A}} = 1$. We may note that $\boldsymbol{\phi}_{\mathrm{o}}\boldsymbol{G}_{\mathscr{A}\mathscr{F}}$, in (4.2), is a $(1 \times k_{\mathscr{F}})$ vector that similarly gives the equilibrium probabilities that a shut period starts in each of the $k_{\mathscr{F}}$ shut states. This we may denote as $\boldsymbol{\phi}_{\mathrm{s}}$, thus

$$\boldsymbol{\phi}_{\mathrm{s}} = \boldsymbol{\phi}_{\mathrm{o}}\boldsymbol{G}_{\mathscr{A}\mathscr{F}}, \tag{6.4}$$

where the subscript stands for shut. The elements of this also sum to unity because $\boldsymbol{G}_{\mathscr{A}\mathscr{F}}$ has rows that sum to unity, $\boldsymbol{G}_{\mathscr{A}\mathscr{F}}\boldsymbol{u}_{\mathscr{F}} = \boldsymbol{u}_{\mathscr{A}}$, so $\boldsymbol{\phi}_{\mathrm{s}}\boldsymbol{u}_{\mathscr{F}} = \boldsymbol{\phi}_{\mathrm{o}}\boldsymbol{G}_{\mathscr{A}\mathscr{F}}\boldsymbol{u}_{\mathscr{F}} = 1$.

The initial vector at $t = 0$. The equilibrium initial vector $\boldsymbol{\phi}_{\mathrm{o}}$ applies to the set of times at which a channel happens to open. In the case of a perturbation applied at $t = 0$, the initial vector must be calculated differently. If, for example, a channel is observed to be open at $t = 0$ then the probability that this particular opening starts in each of the open states (the start being defined to occur at $t = 0$) is simply the occupancy of each of the open states (relative to the total occupancy of open states). Thus if the occupancies of each state at $t = 0$ are represented by the vector $\boldsymbol{p}(0)$, which is partitioned into open and shut states thus

$$\boldsymbol{p}(0) = [\boldsymbol{p}_{\mathscr{A}}(0) \quad \boldsymbol{p}_{\mathscr{F}}(0)], \tag{6.5}$$

then the required initial vector, denoted $\boldsymbol{\phi}_{\mathscr{A}}(0)$ say, is

$$\boldsymbol{\phi}_{\mathscr{A}}(0) = \boldsymbol{p}_{\mathscr{A}}(0)/\boldsymbol{p}_{\mathscr{A}}(0)\,\boldsymbol{u}_{\mathscr{A}}. \tag{6.6}$$

The scalar in the denominator is just the total occupancy of all open states, which is required to ensure that the initial vector sums to unity, i.e.

$$\boldsymbol{\phi}_{\mathscr{A}}(0)\,\boldsymbol{u}_{\mathscr{A}} = 1. \tag{6.7}$$

If the system is at equilibrium before the perturbation is applied then the initial occupancies, $\boldsymbol{p}(0)$, will simply be the equilibrium occupancies, $\boldsymbol{p}(\infty)$, calculated from the rate constants appropriate to the prejump conditions. These can be calculated according to (1.25).

Distributions after a perturbation when the channel was open at $t = 0$

We wish to know the distribution of the length of the nth opening, or the nth shut period after $t = 0$. The definition of n when the channel happened to be open at $t = 0$ is illustrated in figure 3*a*.

The PDF of the length of the first opening, i.e. the latency until the first shutting is found simply by replacing $\boldsymbol{\phi}_{\mathrm{o}}$ by $\boldsymbol{\phi}_{\mathscr{A}}(0)$ in (6.1), giving

$$\boldsymbol{\phi}_{\mathscr{A}}(0)\exp(\boldsymbol{Q}_{\mathscr{A}\mathscr{A}}t)(-\boldsymbol{Q}_{\mathscr{A}\mathscr{A}})\,\boldsymbol{u}_{\mathscr{A}}.$$

Before the second opening is reached there must be a transition from open ($\mathscr{A}$) to shut ($\mathscr{F}$) and back; these transitions are described by the probability transition

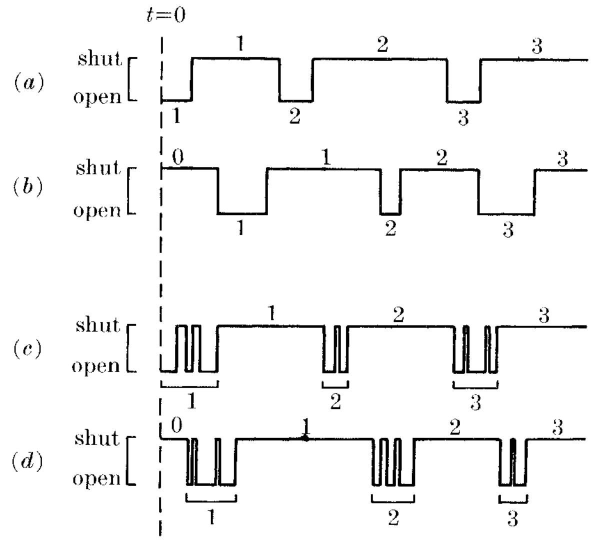


FIGURE 3. The numbering of open and shut periods, and of bursts, after a perturbation at $t = 0$. The numbering of open and shut times is shown in (*a*) and (*b*). The numbering of bursts and of gaps between bursts is shown in (*c*) and (*d*). In (*a*) and (*c*) the channel was open at $t = 0$; in (*b*) and (*d*) the channel was shut at $t = 0$.

matrix $\mathbf{X}_{\mathscr{A}\mathscr{A}} = \mathbf{G}_{\mathscr{A}\mathscr{F}}\,\mathbf{G}_{\mathscr{F}\mathscr{A}}$ (see (1.8)). Thus the distribution of the length of the nth open or shut period (defined in figure 3*a*), given that the channel was open at $t = 0$, is given by replacing $\boldsymbol{\phi}_{\text{o}}$ in (6.1) or (6.2) by the new initial vector

$$\boldsymbol{\phi}_{\mathscr{A}}(0)\,\mathbf{X}^{n-1}_{\mathscr{A}\mathscr{A}}, \quad n \geqslant 1. \tag{6.8}$$

Reduction to the equilibrium distribution. Under certain circumstances the distributions defined by (6.8) reduce to the usual equilibrium distributions in (6.1) and (6.2). (*a*) As would be expected, the equilibrium distribution is always approached after a large number of transitions have occurred because the vector in (6.8) approaches the equilibrium initial vector, $\boldsymbol{\phi}_{\text{o}}$, as $n \to \infty$, as shown by (2.9). (*b*) If the lengths of openings are uncorrelated, i.e. $R(\mathbf{X}_{\mathscr{A}\mathscr{A}}) = 1$ as discussed in §2, then, from (2.13),

$$\mathbf{X}^{n-1}_{\mathscr{A}\mathscr{A}} = \mathbf{u}_{\mathscr{A}}\,\boldsymbol{\phi}_{\text{o}}, \quad n \geqslant 2 \tag{6.9}$$

so all open time distributions *except* that for the first latency will be the same as the equilibrium distribution. Also, the distributions of all shut times, *including* the first, will be the same as the equilibrium shut time distribution because when $R(\mathbf{X}_{\mathscr{A}\mathscr{A}}) = 1$ it will usually be true that $\mathbf{G}_{\mathscr{A}\mathscr{F}} = \mathbf{u}_{\mathscr{A}}\,\boldsymbol{\phi}_{\text{S}}$, so $\boldsymbol{\phi}_{\mathscr{A}}(0)\,\mathbf{G}_{\mathscr{A}\mathscr{F}} = \boldsymbol{\phi}_{\text{S}} = \boldsymbol{\phi}_{\text{o}}\,\mathbf{G}_{\mathscr{A}\mathscr{F}}$ (see (2.18) and (6.4)). (*c*) If there is only one open state ($k_{\mathscr{A}} = 1$), or only one shut state ($k_{\mathscr{F}} = 1$) then the durations of openings and shuttings must be uncorrelated so the results just described under (*b*) will apply.

Distributions after a perturbation when the channel was shut at $t = 0$

The most convenient definition of n when the channel happens to be shut at $t = 0$ is shown in figure 3*b* (the only difference between this and figure 3*a* is that the initial shut period, i.e. the latency to the first opening, is considered separately). In this case we may, in analogy with (6.6), define an initial vector, $\boldsymbol{\phi}_{\mathscr{F}}(0)$, that contains the relative occupancies of each shut state at $t = 0$. Thus

$$\boldsymbol{\phi}_{\mathscr{F}}(0) = \boldsymbol{p}_{\mathscr{F}}(0)/\boldsymbol{p}_{\mathscr{F}}(0)\,\boldsymbol{u}_{\mathscr{F}}. \tag{6.10}$$

The first latency. The latent period until the first opening ($n = 0$, figure 3*b*) will have the PDF

$$f(t) = \boldsymbol{\phi}_{\mathscr{F}}(0)\,\mathrm{e}^{\boldsymbol{Q}_{\mathscr{F}\mathscr{F}}t}(-\boldsymbol{Q}_{\mathscr{F}\mathscr{F}})\,\boldsymbol{u}_{\mathscr{F}}, \quad n = 0. \tag{6.11}$$

All other cases. For all subsequent open and shut periods the PDF can be found by substitution for $\boldsymbol{\phi}_{\mathrm{o}}$, in (6.1) and (6.2) respectively, of the new initial vector

$$\boldsymbol{\phi}_{\mathscr{F}}(0)\,\boldsymbol{G}_{\mathscr{F}\mathscr{A}}\,\boldsymbol{X}_{\mathscr{A}\mathscr{A}}^{n-1}, \quad n \geqslant 1. \tag{6.12}$$

Reduction to the equilibrium distribution. The distributions defined by (6.12) will reduce to the equilibrium distributions, (6.1) and (6.2) under the following circumstances. (*a*) After many transitions ($n \rightarrow \infty$), (6.12) approaches its equilibrium value, $\boldsymbol{\phi}_{\mathrm{o}}$, by virtue of (2.9) and (1.9). (*b*) If openings are uncorrelated all the distributions, except for $n = 0$ in (6.11), reduce to the equilibrium distributions by virtue of (6.9). This is the case even for $n = 1$ because (see (2.18)) when $R(\boldsymbol{G}_{\mathscr{F}\mathscr{A}}) = 1$ we have $\boldsymbol{G}_{\mathscr{F}\mathscr{A}} = \boldsymbol{u}_{\mathscr{F}}\,\boldsymbol{\phi}_{\mathrm{o}}$, so $\boldsymbol{\phi}_{\mathscr{F}}(0)\,\boldsymbol{G}_{\mathscr{F}\mathscr{A}} = \boldsymbol{\phi}_{\mathrm{o}}$ in (6.12). (*c*) If there is only one open state, or one shut state, then openings are uncorrelated so the results just described under (*b*) will apply.

Bursts after a perturbation

We now consider the case where the states of the system are divided into subsets $\mathscr{A}$, $\mathscr{B}$ and $\mathscr{C}$ as defined in §1.

The equilibrium initial vector

The vector $\boldsymbol{\phi}_{\mathrm{b}}$, which gives the equilibrium probabilities that (the first opening of) a burst starts in each of the open states is defined and discussed by Colquhoun & Hawkes (1982, equations (3.2) and (A 1.10)–(A 1.24)). The equilibrium occupancies of the various states of the system at $t = 0$ may be partitioned simply into open and shut states, as already defined in (6.5), or as

$$\boldsymbol{p}(0) = [\boldsymbol{p}_{\mathscr{A}}(0) \quad \boldsymbol{p}_{\mathscr{B}}(0) \quad \boldsymbol{p}_{\mathscr{C}}(0)]. \tag{6.13}$$

We wish to define the distributions of attributes of the nth burst, or of the nth gap-between-bursts, after $t = 0$. The definition of n is illustrated in figure 3*c* for the case where the channel was open at $t = 0$, and in figure 3*d* for the case where the channel was shut at $t = 0$ (the only difference is that in the latter case the initial shut period, i.e. the latency until the first opening, is considered separately). Two versions of the latter case are of interest: (*a*) the case where the channel is simply observed to be shut (in any $\mathscr{F}$ state) at $t = 0$ and (*b*) the case where the channel is known to be in one of the $\mathscr{C}$ states at $t = 0$, so we know that we start in a 'gap between bursts' (this might happen, for example, in the case of an agonist-operated channel when no agonist is present up to $t = 0$ so all channels are in the resting $\mathscr{C}$ state(s) until, at $t = 0$, a low agonist concentration is applied).

A considerable number of different sorts of distributions can be defined for a channel that shows bursting behaviour (e.g. the number of openings per burst, the burst length, the total open time per burst, the length of the kth opening in a burst with r openings, etc. as described by Colquhoun & Hawkes 1982). In each case† the expression for the equilibrium distribution starts with the equilibrium initial vector $\boldsymbol{\phi}_{\mathrm{b}}$. As in the previous case the distributions (and their means) after a perturbation can be found by replacing $\boldsymbol{\phi}_{\mathrm{b}}$ in these expressions by the initial vectors given below.

Results conditional on being open at $t = 0$

The distributions, conditional on the channel being open at $t = 0$, for the nth burst, or the nth gap between bursts (see figure 3c), are given by replacing $\boldsymbol{\phi}_{\mathrm{b}}$ in the corresponding equilibrium expression by

$$\boldsymbol{\phi}_{\mathscr{A}}(0)\,\boldsymbol{Z}_{\mathscr{A}\mathscr{A}}^{n-1}, \quad n \geqslant 1, \tag{6.14}$$

where $\boldsymbol{\phi}_{\mathscr{A}}(0)$ is as described above (see (6.6)), and $\boldsymbol{Z}_{\mathscr{A}\mathscr{A}}$ is the probability transition matrix from the start of one burst to the start of the next (see (1.13)). After sufficient time the equilibrium distributions are approached because $\boldsymbol{Z}_{\mathscr{A}\mathscr{A}}^{n-1}$ approaches $\boldsymbol{u}_{\mathscr{A}}\,\boldsymbol{\phi}_{\mathrm{b}}$ as $n \to \infty$ (see (4.10)). If bursts are uncorrelated, i.e. $R(\boldsymbol{Z}_{\mathscr{A}\mathscr{A}}) = 1$ (see §4) then, from (4.11), $\boldsymbol{Z}_{\mathscr{A}\mathscr{A}} = \boldsymbol{u}_{\mathscr{A}}\,\boldsymbol{\phi}_{\mathrm{b}}$ so (6.14) reduces to $\boldsymbol{\phi}_{\mathrm{b}}$ and we obtain the equilibrium distributions in all cases, except for $n = 1$. If there is only one open state ($k_{\mathscr{A}} = 1$) then $\boldsymbol{\phi}_{\mathscr{A}}(0) = \boldsymbol{\phi}_{\mathrm{b}} = 1$ so we get the equilibrium distribution even for $n = 1$ (i.e. the latency of the first gap between bursts).

Results conditional on being shut at $t = 0$

If we know only that the channel is shut (in a $\mathscr{B}$ state or a $\mathscr{C}$ state) at $t = 0$ an initial vector can be defined, as in (6.10), to give the relative occupancies of each shut state at $t = 0$, namely $\boldsymbol{\phi}_{\mathscr{F}}(0) = \boldsymbol{p}_{\mathscr{F}}(0)/\boldsymbol{p}_{\mathscr{F}}(0)\,\boldsymbol{u}_{\mathscr{F}}$.

The first latency. The distribution of the latency to the start of the first burst ($n = 0$ in figure 3d) is

$$\boldsymbol{\phi}_{\mathscr{F}}(0)\,\mathrm{e}^{\boldsymbol{Q}_{\mathscr{F}\mathscr{F}}t}(-\boldsymbol{Q}_{\mathscr{F}\mathscr{F}})\,\boldsymbol{u}_{\mathscr{F}}, \quad n = 0, \tag{6.15}$$

just as for the simple open–shut case (6.11).

All other cases. The distributions, conditional on the channel being shut at $t = 0$, for the nth burst, or the nth gap between bursts (defined in figure 3d), are found by replacing $\boldsymbol{\phi}_{\mathrm{b}}$ in the corresponding equilibrium expression by

$$\boldsymbol{\phi}_{\mathscr{F}}(0)\,\boldsymbol{G}_{\mathscr{F}\mathscr{A}}\,\boldsymbol{Z}_{\mathscr{A}\mathscr{A}}^{n-1}, \quad n \geqslant 1. \tag{6.16}$$

After sufficient time the equilibrium distributions are approached because $\boldsymbol{Z}_{\mathscr{A}\mathscr{A}}^{n-1}$ approaches $\boldsymbol{u}_{\mathscr{A}}\,\boldsymbol{\phi}_{\mathrm{b}}$ as $n \to \infty$, and $\boldsymbol{G}_{\mathscr{F}\mathscr{A}}\,\boldsymbol{u}_{\mathscr{A}} = \boldsymbol{u}_{\mathscr{F}}$ (see (1.9) and (4.10)). When the bursts are uncorrelated so $R(\boldsymbol{Z}_{\mathscr{A}\mathscr{A}}) = 1$ and $\boldsymbol{Z}_{\mathscr{A}\mathscr{A}} = \boldsymbol{u}_{\mathscr{A}}\,\boldsymbol{\phi}_{\mathrm{b}}$, (6.16) reduces to $\boldsymbol{\phi}_{\mathrm{b}}$ for $n \geqslant 2$. However, for the first burst and gap ($n = 1$ in figure 3d), (6.16) will reduce to $\boldsymbol{\phi}_{\mathrm{b}}$ only if $R(\boldsymbol{G}_{\mathscr{F}\mathscr{A}}) = 1$ so *openings* are uncorrelated also; in this case $\boldsymbol{G}_{\mathscr{F}\mathscr{A}} = \boldsymbol{u}_{\mathscr{F}}\,\boldsymbol{\phi}_{\mathrm{o}} = \boldsymbol{u}_{\mathscr{F}}\,\boldsymbol{\phi}_{\mathrm{b}}$ as used above (see (2.18)). Note that, although uncorrelated openings imply uncorrelated bursts, the converse is not necessarily true.

† The final form of the distribution of gaps between bursts is given by Colquhoun & Hawkes (1982, equation (3.85)) in a form that starts with a vector denoted $\boldsymbol{\psi}_{\mathrm{g}}$. However, comparison of their equations (3.82) and (3.83) shows that $\boldsymbol{\psi}_{\mathrm{g}} = \boldsymbol{\phi}_{\mathrm{b}}(\boldsymbol{I} - \boldsymbol{H}_{\mathscr{A}\mathscr{A}})^{-1}(-\boldsymbol{Q}_{\mathscr{A}\mathscr{A}}^{-1})$, so this distribution too can be written starting with $\boldsymbol{\phi}_{\mathrm{b}}$.

Results conditional on being in $\mathscr{C}$ at $t = 0$

In this case the relative occupancies at $t = 0$ are given (see (6.13)) by

$$\boldsymbol{\phi}_{\mathscr{C}}(0) = \boldsymbol{p}_{\mathscr{C}}(0)/\boldsymbol{p}_{\mathscr{C}}(0)\,\boldsymbol{u}_{\mathscr{C}}. \tag{6.17}$$

The first latency. The PDF of the latency to the start of the first burst ($n = 0$ in figure 3*d*) will have Laplace transform

$$f^*(s) = \boldsymbol{\phi}_{\mathscr{C}}(0)\,[\boldsymbol{I} - \boldsymbol{G}^*_{\mathscr{C}\mathscr{B}}(s)\,\boldsymbol{G}^*_{\mathscr{B}\mathscr{C}}(s)]^{-1}[\boldsymbol{G}^*_{\mathscr{C}\mathscr{B}}(s)\,\boldsymbol{G}^*_{\mathscr{B}\mathscr{A}}(s) + \boldsymbol{G}^*_{\mathscr{C}\mathscr{A}}(s)]\,\boldsymbol{u}_{\mathscr{A}}, \tag{6.18}$$

(this follows from the principles used to derive the distribution of gaps between bursts, equation (3.82) in Colquhoun & Hawkes (1982), but in this case there is no possibility of an initial silent period in $\mathscr{B}$). The inverse of this gives the required PDF, which can be shown to be

$$f(t) = \boldsymbol{\phi}_{\mathscr{C}}(0)\,[\boldsymbol{P}_{\mathscr{F}\mathscr{F}}(t)]_{\mathscr{C}\mathscr{F}}\,\boldsymbol{Q}_{\mathscr{F}\mathscr{A}}\,\boldsymbol{u}_{\mathscr{A}}, \quad (n = 0). \tag{6.19}$$

The expression in square brackets is the '$\mathscr{C}\mathscr{F}$' subsection (i.e. the last $k_{\mathscr{C}}$ rows) of $\boldsymbol{P}_{\mathscr{F}\mathscr{F}}(t) = \exp(\boldsymbol{Q}_{\mathscr{F}\mathscr{F}}\,t)$. This is an intuitively reasonable form to describe a sojourn in $\mathscr{F}$ that starts in $\mathscr{C}$ and eventually exits from any $\mathscr{F}$ state to $\mathscr{A}$.

All other cases. The distributions, conditional on the channel being in $\mathscr{C}$ at $t = 0$, for the nth burst, or gap between bursts, are given when $n \geqslant 1$ (see figure 3*d*) by replacing $\boldsymbol{\phi}_{\mathrm{b}}$ in the corresponding equilibrium expressions by

$$\boldsymbol{\phi}_{\mathscr{C}}(0)\,\boldsymbol{G}_{\mathscr{C}(\mathscr{B})\mathscr{A}}\,\boldsymbol{Z}^{n-1}_{\mathscr{A}\mathscr{A}}, \quad n \geqslant 1, \tag{6.20}$$

where $\boldsymbol{G}_{\mathscr{C}(\mathscr{B})\mathscr{A}}$ was defined in (1.12). The equilibrium distribution will be approached when $n \to \infty$, as before (see (6.14), (4.10) and (1.14)). When bursts are uncorrelated, so $R(\boldsymbol{Z}_{\mathscr{A}\mathscr{A}}) = R(\boldsymbol{G}_{\mathscr{C}(\mathscr{B})\mathscr{A}}) = 1$, (6.20) will become $\boldsymbol{\phi}_{\mathrm{b}}$ so that the distributions (for $n \geqslant 1$) will become identical with the equilibrium distributions, because in this case $\boldsymbol{Z}_{\mathscr{A}\mathscr{A}} = \boldsymbol{u}_{\mathscr{A}}\,\boldsymbol{\phi}_{\mathrm{b}}$, and $\boldsymbol{G}_{\mathscr{C}(\mathscr{B})\mathscr{A}} = \boldsymbol{u}_{\mathscr{C}}\,\boldsymbol{\phi}_{\mathrm{b}}$.

The first latency distribution when there are N channels present

Under conditions where there are no correlations, so that it is predicted that all distributions will be the same regardless of n, the distributions of open-time and of 'within-burst' characteristics should be close to the distributions given above even when there are several channels rather than just one as was assumed in all the foregoing results. However, only in the case of the first latency have we obtained exact results.

The distribution of the first latency for one channel, given above, can easily be generalized to take account of the presence of any number, N say, of channels, as long as the channels behave independently of one another. Define $f_1(t)$ as the PDF of the first latency for a single channel, and $f_N(t)$ as the PDF of the first latency when N independent channels are present. Various sorts of first latency distributions have been defined above, but all of them have the general form

$$f_1(t) = \sum a_i\,\lambda_i\,\mathrm{e}^{-\lambda_i t}, \tag{6.21}$$

where $\sum a_i = 1$. The number of components, the areas (a_i) and the rates (λ_i) will depend on the particular problem. Thus the probability that the latency is *greater* than t, for one channel, is

$$P(\text{latency} > t) = 1-F_1(t) = \int_t^\infty f_1(t)\,\mathrm{d}t,$$
$$= \sum a_i\,\mathrm{e}^{\lambda_i t}, \tag{6.22}$$

where $F_1(t)$ is the distribution function for the first latency for one channel. When N independent channels are present, the observed first latency will be greater than t if the first latencies for all N individual channels are greater than t so

$$P(\text{all } N \text{ latencies} > t) = 1-F_N(t) = [1-F_1(t)]^N. \tag{6.23}$$

This expression was used by Aldrich *et al.* (1983). The required PDF, the derivative of $F_N(t)$, is thus

$$f_N(t) = N[1-F_1(t)]^{N-1}f_1(t). \tag{6.24}$$

The case of a single exponential. If the distribution of first latency for one channel (6.21) has only one exponential component so it has the form $f_1(t) = \lambda\,\mathrm{e}^{-\lambda t}$, with mean $\tau = 1/\lambda$, then (6.24) becomes

$$f_N(t) = N\lambda\,\mathrm{e}^{-N\lambda t}. \tag{6.25}$$

This is a simple exponential distribution with mean $1/N\lambda = \tau/N$, so the mean latency is reduced by a factor of N, compared with that for one channel.

The case of two exponentials. When $f_1(t)$ has the form $a_1\,\lambda_1\,\mathrm{e}^{-\lambda_1 t}+a_2\,\lambda_2\,\mathrm{e}^{-\lambda_2 t}$ (where $\lambda_1 > \lambda_2$, say) the mean first latency is $\mu_1 = a_1\,\tau_1+a_2\,\tau_2$ for one channel. The distribution for N channels follows from (6.24); the mean first latency, μ_N, from the binomial expansion, is

$$\mu_N = \sum_{r=0}^{N}\binom{N}{r}\frac{a_1^{N-r}\,a_2^r}{(N-r)\,\lambda_1+r\lambda_2}. \tag{6.26}$$

The meaning of this result may be illustrated by the first term, which is $a_1^N/N\lambda_1$. A fraction of a_1^N of all observed first latencies will be such that the latency for each of the N channels is of the short type (mean $\tau_1 = 1/\lambda_1$); the mean latency for these will be $1/N\lambda_1 = \tau_1/N$. If $\lambda_1 \gg \lambda_2$ this term will predominate, i.e. the observed latency will be largely dictated by the shorter time constant.

Numerical example of distributions after a concentration jump

This example is based on **6** in figure 2, and using the rate constants suggested by Colquhoun & Sakmann (1985) in their attempt to fit observations made with suberyldicholine on frog end-plate channels. The scheme is thus:

$$\begin{array}{ccccc}
5 & \mathrm{R} & & & \\
 & \Big\updownarrow\ 2410\ \mathrm{s^{-1}} & & & \\
4 & \mathrm{AR} & \underset{6250\ \mathrm{s^{-1}}}{\overset{0.72\ \mathrm{s^{-1}}}{\rightleftharpoons}} & \mathrm{AR^*} & 1 \\
 & \Big\updownarrow\ 2410\ \mathrm{s^{-1}} & & \Big\updownarrow\ 0.01\ \mathrm{s^{-1}} & \\
3 & \mathrm{A_2R} & \underset{625\ \mathrm{s^{-1}}}{\overset{18000\ \mathrm{s^{-1}}}{\rightleftharpoons}} & \mathrm{A_2R^*} & 2
\end{array} \tag{6.27}$$

where A denotes agonist, and the receptor channel is denoted R (shut) and R* (open). All the association rate constants were taken as $1.6 \times 10^8\ \text{M}^{-1}\ \text{s}^{-1}$. Calculations have been done on the distributions expected following a jump in concentration of agonist from zero to 4 nM. The initial state is therefore that all channels are in state 5 before $t = 0$. There is no correlation between bursts in this case (see §4) so all the burst distributions are identical wherever they occur after the concentration jump; they are the equilibrium distributions (some of which are given by Colquhoun & Sakmann 1985). The only exception is the distribution of the shut time up to start of the first burst (the first latency), which is the same as that given in table 2 for the latency to the first opening.

Open times are correlated for (6.27), so the distributions of open and shut times will depend on where they occur after the concentration jump. In this case $\rho(1) = 0.073$ for open times, 0.175 for shut times and -0.166 for the open–shut correlation. The distributions are summarized in table 2, which gives the area for each component of (*a*) the open-time distribution and (*b*) the shut-time distribution for the *n*th event (see figure 2*a*) following the concentration jump. All channels are in the resting state at $t = 0$ so the distributions conditional on being shut are $t = 0$ are the only relevant ones (see (6.10)–(6.12)).

The initial vectors are also shown in table 2. They give, for the open-time distributions, the probability that the *n*th opening starts in each of the open states

TABLE 2. OPEN- AND SHUT-TIME DISTRIBUTIONS FOLLOWING A CONCENTRATION JUMP FROM 0 TO 4 nM, FOR THE SCHEME IN (6.27).

(The numbering, n, of the openings and shuttings is as in figure 3(*b*) because the channel must be shut at $t = 0$. The initial vector gives the probability that an interval starts in the specified state. See text for details.)

(*a*) *Open-time distribution*

	initial vector		area	
n	1	2	$\tau = 0.16$ ms	$\tau = 1.6$ ms
1	0.588	0.412	0.588	0.412
2	0.397	0.603	0.397	0.603
3	0.308	0.692	0.308	0.692
4	0.267	0.733	0.267	0.733
5	0.248	0.752	0.248	0.752
6	0.239	0.761	0.239	0.761
⋮				
∞	0.232	0.768	0.231	0.769

(*b*) *Shut-time distribution*

	initial vector			area		
n	3	4	5	$\tau = 43.8$ μs	$\tau = 0.415$ ms	$\tau = 1538.8$ s
0	0	0	1	0	0	1
1	0.412	0.588	0	0.325	0.0004	0.675
2	0.603	0.397	0	0.476	0.0003	0.524
3	0.692	0.308	0	0.546	0.0003	0.454
4	0.733	0.267	0	0.578	0.0002	0.422
5	0.752	0.248	0	0.593	0.0002	0.407
6	0.761	0.239	0	0.600	0.0002	0.400
⋮						
∞	0.768	0.232	0	0.606	0.0002	0.394

(states 1 and 2), namely $\boldsymbol{\phi}_{\mathscr{F}}(0)\,\boldsymbol{G}_{\mathscr{F}\mathscr{A}}\,\boldsymbol{X}^{n-1}_{\mathscr{A}\mathscr{A}}$, from (6.1) and (6.12). For the shut-time distributions the initial vector gives the probability that the nth shut time starts in each of the shut states (states 3, 4 and 5); from (6.2), (6.11) and (6.12) this is seen to be $\boldsymbol{\phi}_{\mathscr{F}}(0)\,\boldsymbol{G}_{\mathscr{F}\mathscr{A}}\,\boldsymbol{X}^{n-1}_{\mathscr{A}\mathscr{A}}\,\boldsymbol{G}_{\mathscr{A}\mathscr{F}} = \boldsymbol{\phi}_{\mathscr{F}}(0)\,(\boldsymbol{G}_{\mathscr{F}\mathscr{A}}\,\boldsymbol{G}_{\mathscr{A}\mathscr{F}})^n$. At $t = 0$ all channels are in state 5 so $\boldsymbol{\phi}_{\mathscr{F}}(0) = [0\ \ 0\ \ 1]$. Thereafter the initial vector approaches its equilibrium value, as described above.

The open-time distributions show far more 'short openings' just after the jump than at equilibrium; this is to be expected because after leaving R (state 5) at $t = 0$ it is necessary to pass through AR (state 4), from which short openings can originate directly, before any other states are reached. There is, in this example, little interchange between the two open states so the time constants of the open-time distribution (0.16 and 1.6 ms) are close to the mean lifetimes of AR* and A_2R^*, respectively, and the relative frequencies (areas) in the distribution are close to the probabilities, in the initial vector, that an opening starts in each of these states. Also the probability that an opening starts in A_2R^* (state 2) is very close to the probability that a shut period starts in A_2R (state 3).

The shut-time distribution shows a first latency that consists almost entirely of the slowest component of the shut-time distribution ($\tau = 1538.8$ s); it is virtually a single exponential so (6.25) would apply if there were more than one channel present. Shut periods after the first must start in A_2R or AR (states 3 and 4). The former becomes more common as the equilibrium occupancy of doubly occupied channels is approached, when 76.8% of shut periods start with an $A_2R^* \rightarrow A_2R$ transition (as shown by the initial vector in table 2), and brief shut periods (spent mainly in A_2R) become concomitantly more common than they were near $t = 0$.

7. Components with zero area in the distribution of the number of openings per burst

In principle, the distribution of the number of openings per burst has a number of geometric components equal to the number of open states, $k_{\mathscr{A}}$ (Colquhoun & Hawkes 1982). However, in some circumstances there may be fewer components than this because the areas of some components (which have unit mean) may be zero. Thus the existence or non-existence of a discrepancy between the number of components in this distribution, and the number of exponential components in the distribution of open times (or, often more unambiguously, in the distribution of the total open time per burst) which should also both be $k_{\mathscr{A}}$, may give clues about mechanisms (see, for example, Jackson *et al.* 1983; Colquhoun & Sakmann 1985).

Distribution of the number of openings per burst

The probability of observing r openings per burst was given by Colquhoun & Hawkes (1982, equations (3.5) and (3.9)) as

$$\begin{aligned} P(r) &= \boldsymbol{\phi}_{\mathrm{b}}\,\boldsymbol{H}^{r-1}_{\mathscr{A}\mathscr{A}}(\boldsymbol{I}-\boldsymbol{H}_{\mathscr{A}\mathscr{A}})\,\boldsymbol{u}_{\mathscr{A}}, \\ &= \sum_{m=1}^{k_{\mathscr{A}}} a_m(1-\lambda_m)\,\lambda_m^{r-1}, \quad r = 1, 2, \ldots, \end{aligned} \tag{7.1}$$

where λ_m denotes the eigenvalues of $\boldsymbol{H}_{\mathscr{A}\mathscr{A}} = \boldsymbol{G}_{\mathscr{A}\mathscr{B}}\,\boldsymbol{G}_{\mathscr{B}\mathscr{A}}$ and a_m represents the area of each of the $k_{\mathscr{A}}$ geometric components that we have written explicitly in the second form above (for which see Colquhoun & Sigworth 1983, equation (64)). The 'mean number of openings per burst', μ_m, for each component (analogous with the time constants for exponentials) is

$$\mu_m = 1/(1-\lambda_m), \tag{7.2}$$

and the overall mean, which can be written as the weighted mean of the μ_m, is

$$\boldsymbol{\phi}_{\mathrm{b}}(\boldsymbol{I}-\boldsymbol{H}_{\mathscr{A}\mathscr{A}})^{-1}\boldsymbol{u}_{\mathscr{A}} = \sum_{m=1}^{k_{\mathscr{A}}} a_m \mu_m. \tag{7.3}$$

The areas, a_m, of the components can be written in terms of $\boldsymbol{A}_m$, the spectral expansion matrices for $\boldsymbol{H}_{\mathscr{A}\mathscr{A}}$ (see (1.5)), as

$$a_m = \boldsymbol{\phi}_{\mathrm{b}}\boldsymbol{A}_m\boldsymbol{u}_{\mathscr{A}}. \tag{7.4}$$

Components with unit mean

If $\boldsymbol{H}_{\mathscr{A}\mathscr{A}}$ has less than full rank (i.e. rank less than $k_{\mathscr{A}}$), then some of its eigenvalues will be zero and, from (7.2), for each such eigenvalue there is a component with mean $\mu = 1$. We therefore expect to see an 'excess' of isolated openings (i.e. of bursts that contain only one opening). The physical significance of this is discussed further below. The number of zero eigenvalues will generally be equal to $k_{\mathscr{A}} - R(\boldsymbol{H}_{\mathscr{A}\mathscr{A}})$, the nullity of $\boldsymbol{H}_{\mathscr{A}\mathscr{A}}$ (see §1). Even when there is more than one zero eigenvalue the usual spectral expansion appears to hold, and Jordan forms are not needed (we have, so far, no general proof of this proposition).

In certain cases the area for the component(s) with unit mean is predicted to be zero so the unit mean component will *not* be seen, and the number of components will be less than $k_{\mathscr{A}}$ in *principle* (for any mechanism it is, of course, always possible that some components may be too small to be detectable in practice).

The physical significance of the excess of isolated openings is that they consist of a transition from a $\mathscr{C}$ state to an $\mathscr{A}$ state and then back to a $\mathscr{C}$ state (a direct return to $\mathscr{C}$ will end the burst); this component will be absent (have zero area) whenever there are no direct routes from the $\mathscr{A}$ states to the $\mathscr{C}$ states (so $\boldsymbol{Q}_{\mathscr{A}\mathscr{C}} = \boldsymbol{0}$) as in the examples in figure 2, schemes **4**, **8** and **12**. When direct routes from $\mathscr{A}$ to $\mathscr{C}$ do exist then a component with $\mu = 1$ *may* be apparent. For example, in figure 2 many schemes (**2**, **3**, **5**, **7**, **13**, **14**, **16–18** and **23**), which have $\mathscr{A}$–$\mathscr{C}$ routes, do show such a component. However, **1**, **9–11**, **21** and **22** in figure 2, which also have $\mathscr{A}$–$\mathscr{C}$ routes, nevertheless have zero area for the $\mu = 1$ component. The precise conditions under which the component with unit mean appears are considered below, after some examples have been discussed.

Three simple cases

The foregoing argument can be illustrated by comparison of the three simple schemes shown in figure 2, **1–3**. These are reproduced here for convenience.

$$\begin{array}{ccc} & & \mathrm{C}_4 \\ & & \updownarrows \\ \mathrm{B}_3 & \rightleftharpoons & \mathrm{A}_1 \\ & & \updownarrows \\ & & \mathrm{A}_2 \end{array} \qquad\qquad \begin{array}{ccc} & & \mathrm{C}_4 \\ & & \updownarrows \\ & & \mathrm{A}_1 \\ & & \updownarrows \\ \mathrm{B}_3 & \rightleftharpoons & \mathrm{A}_2 \end{array} \qquad\qquad \begin{array}{ccc} & & \mathrm{C}_4 \\ & & \updownarrows \\ \mathrm{B}_3 & \rightleftharpoons & \mathrm{A}_1 \\ \updownarrows & & \\ \mathrm{A}_2 & & \end{array}$$

1 **2** **3**

All of these schemes have two open states ($k_{\mathscr{A}} = 2$), but the direct connectivity between $\mathscr{A}$ and $\mathscr{B}$ is $D^{\mathrm{v}}(\mathscr{A}, \mathscr{B}) = 1$ so $R(\boldsymbol{H}_{\mathscr{A}\mathscr{A}}) = 1$ for all of them (it cannot be larger because $k_{\mathscr{B}} = 1$). Therefore all three will have a component with unit mean ($\mu = 1$) in the distribution of the number of openings per burst. In addition all three schemes have direct routes from $\mathscr{A}$ and $\mathscr{C}$. However, **1** has $R(\boldsymbol{X}_{\mathscr{A}\mathscr{A}}) = 1$; there is a single gateway state, A_1, between open ($\mathscr{A}$) and shut ($\mathscr{F}$) states, whereas **2** and **3** both have $C^{\mathrm{v}}(\mathscr{A}, \mathscr{F}) = 2$ so for both $R(\boldsymbol{X}_{\mathscr{A}\mathscr{A}}) = 2$. Also, **1** has zero area for the component with unit mean whereas **2** and **3** have not.

It will be useful in discussion of these examples to use the transition probability π_{ij}, which gives the probability that a channel in state i moves next to state j (regardless of how long elapses before the transition occurs) as

$$\pi_{ij} = q_{ij}/(-q_{ii}). \tag{7.5}$$

(The $\boldsymbol{G}$ matrices are generalizations of such transition probabilities that are used when subsets of states rather than single states are involved.) The denominator in (7.5) is simply the sum of all rate constants for exit from state i.

A numerical example for **1**

Suppose that the rate constants in **1** are $q_{14} = 500\ \mathrm{s}^{-1}$, $q_{41} = 50\ \mathrm{s}^{-1}$, $q_{13} = 2000\ \mathrm{s}^{-1}$, $q_{31} = 20000\ \mathrm{s}^{-1}$, $q_{12} = 2500\ \mathrm{s}^{-1}$, $q_{21} = 1000\ \mathrm{s}^{-1}$, then the mean lifetimes of stays in individual states are 20 ms for C_4, 50 μs for B_3, 0.2 ms for A_1 and 1 ms for A_2. An average opening will contain one (equivalent to $q_{12}/(q_{13}+q_{14})$) sojourn in A_2 and therefore two sojourns in A_1; the mean length of a single opening is therefore 1.4 ms. The components of the distribution of the number of openings per burst have means of $\mu_1 = 5$ and $\mu_2 = 1$, from (7.2); i.e. the eigenvalues of $\boldsymbol{H}_{\mathscr{A}\mathscr{A}}$ are $\lambda_1 = q_{13}/(q_{13}+q_{14}) = 0.8$, and $\lambda_2 = 0$ because $(\lambda_1+\lambda_2) = \mathrm{Tr}\,(\boldsymbol{H}_{\mathscr{A}\mathscr{A}}) = 0.8$ and $\lambda_1\lambda_2 = \det(\boldsymbol{H}_{\mathscr{A}\mathscr{A}}) = 0$. We find that

$$\boldsymbol{G}_{\mathscr{A}\mathscr{B}} = \begin{bmatrix} 0.8 \\ 0.8 \end{bmatrix}, \quad \boldsymbol{G}_{\mathscr{B}\mathscr{A}} = [1 \quad 0], \quad \boldsymbol{H}_{\mathscr{A}\mathscr{A}} = \begin{bmatrix} 0.8 & 0 \\ 0.8 & 0 \end{bmatrix}, \tag{7.6}$$

and, because $\lambda_2 = 0$, $\boldsymbol{H}_{\mathscr{A}\mathscr{A}} = \boldsymbol{A}_1\lambda_1$ so

$$\boldsymbol{A}_1 = \boldsymbol{H}_{\mathscr{A}\mathscr{A}}/\lambda = \begin{bmatrix} 1 & 0 \\ 1 & 0 \end{bmatrix}; \quad \boldsymbol{A}_2 = \boldsymbol{I} - \boldsymbol{A}_1 = \begin{bmatrix} 0 & 0 \\ -1 & 1 \end{bmatrix}. \tag{7.7}$$

All bursts must start in state 1 so $\boldsymbol{\phi}_{\mathrm{b}} = [1 \quad 0]$. Thus the areas of the two components in the distribution of the number of openings per burst, from (7.4), are $a_1 = 1$, $a_2 = 0$. In other words, there will be only a *single* geometric component with a mean of five openings per burst. The existence of two open states would be indetectable from this distribution. However, the distribution of open times has, in this case, two quite clear components, with time constants of 2.22 ms (59.8% of area) and 0.18 ms (40.2% of area). These values are not surprising as 50% of openings consist of a single sojourn in A_1 (with mean length 0.2 ms). Openings (and hence also bursts) are uncorrelated because $R(\boldsymbol{X}_{\mathscr{A}\mathscr{A}}) = 1$ so all open times, wherever they occur in a burst, will have the same distribution.

A numerical example for **2**

The foregoing results may be contrasted with those for **2** with $q_{14} = 3500\ \mathrm{s}^{-1}$, $q_{41} = 50\ \mathrm{s}^{-1}$, $q_{23} = 736.8\ \mathrm{s}^{-1}$, $q_{32} = 20000\ \mathrm{s}^{-1}$, $q_{12} = 1500\ \mathrm{s}^{-1}$, $q_{21} = 263.2\ \mathrm{s}^{-1}$. The mean lifetimes are therefore 20 ms for C_4, 50 μs for B_3, 0.2 ms for A_1 and 1 ms for A_2, as in the previous example. Because $R(\boldsymbol{X}_{\mathscr{A}\mathscr{A}}) = 2$ openings will be correlated (see below); the overall mean length of a single opening is 0.871 ms. The components of the distribution of the number of openings per burst will, as in the previous case, have means of $\mu_1 = 5$ and $\mu_2 = 1$ (where $\mu_1 = 1+q_{23}/(q_{21}\pi_{14})$), $\pi_{14} = q_{14}/(q_{12}+q_{14})$, i.e. the eigenvalues of $\boldsymbol{H}_{\mathscr{A}\mathscr{A}}$ are again $\lambda_1 = 0.8$, $\lambda_2 = 0$. However, in this case the component with $\mu_2 = 1$ has not got zero area, but 70 % of the area. We have $a_2 = \pi_{14} = 0.7$; i.e. the area for the unit mean component is simply the probability that a channel in state A_1 will, at its next transition, go back to $\mathscr{C}$, rather than proceeding to A_2. More formally, the matrices involved are as follows.

$$\boldsymbol{G}_{\mathscr{A}\mathscr{B}} = \begin{bmatrix} 0.24 \\ 0.8 \end{bmatrix}, \quad \boldsymbol{G}_{\mathscr{B}\mathscr{A}} = [0 \quad 1], \quad \boldsymbol{H}_{\mathscr{A}\mathscr{A}} = \begin{bmatrix} 0 & 0.24 \\ 0 & 0.8 \end{bmatrix},$$

$$\boldsymbol{A}_1 = \begin{bmatrix} 0 & 0.3 \\ 0 & 1 \end{bmatrix}, \quad \boldsymbol{A}_2 = \begin{bmatrix} 1 & -0.3 \\ 0 & 0 \end{bmatrix}. \tag{7.8}$$

Again $\boldsymbol{\phi}_b = [1 \quad 0]$ so, from (7.4), the areas are $a_1 = 0.3$, $a_2 = 0.7$, as stated above. The component with unit mean is very prominent; 76 % of *all* bursts have only one opening.

In this case $R(\boldsymbol{X}_{\mathscr{A}\mathscr{A}}) = 2$ so open times are correlated. We find that

$$\boldsymbol{X}_{\mathscr{A}\mathscr{A}} = \begin{bmatrix} 0.76 & 0.24 \\ 0.20 & 0.80 \end{bmatrix}, \tag{7.9}$$

with eigenvalues of 1 and 0.56. From (2.11) we find the correlation coefficients to be $\rho(1) = 0.083$; $\rho(2) = 0.046$, $\rho(3) = 0.026, \ldots$. As expected from (2.11) they decay in a simple geometric fashion with a ratio of 0.56 between successive values. The correlations (if there is only one channel) between shut times, from (2.17), are $\rho(1) = 0.197$, $\rho(2) = 0.110$, $\rho(3) = 0.062, \ldots$; and the open–shut correlations, from (2.20) are $\rho(1) = -0.171$, $\rho(2) = -0.096$, $\rho(3) = -0.054, \ldots$. There is no correlation within or between bursts because $R(\boldsymbol{H}_{\mathscr{A}\mathscr{A}}) = 1$ and $R(\boldsymbol{Z}_{\mathscr{A}\mathscr{A}}) = 1$.

The distribution of open times has time constants of 0.196 ms and 1.11 ms, quite close to the mean lifetimes of the two open states A_1 and A_2 (namely 0.2 and 1 ms). However, as expected from the correlation between open times, the relative areas of these components will depend on the position of the opening in a burst. As might be expected from the discussion above, bursts with only one opening consist predominantly of a single sojourn in A_1 (mean life 0.2 ms); and the distribution of the open time in such bursts has 88.3 % of the area for the component with $\tau = 0.196$ ms. For bursts with more than one opening it is necessary, at the start of the burst, to pass through both A_1 and A_2 to reach B_3 (the gap-within-burst state), and to reverse this route to return to C_4 at the end of the burst. The first and last opening of any burst with more than one opening will have a distribution quite close to that expected for the *sum* of lengths of a sojourn in A_1 and in A_2.

The mean of this distribution would clearly be 1.2 ms, and the probability density function would have (see, for example, Colquhoun & Hawkes 1983, pp. 165–167) time constants of 1 ms (area = 1.25) and 0.2 (area = −0.25). This is quite close to the actual calculated distribution, which has area = 1.22 for $\tau = 1.11$ ms, and area = −0.22 for $\tau = 0.196$ ms. The mean is 1.303 ms (longer than 1.2 ms because in a fraction $0.079 = \pi_{21}\pi_{12}$ of cases there will be $A_2 \rightarrow A_1 \rightarrow A_2$ oscillations before reaching B_3). This distribution goes through a peak; short durations will be rare because it is necessary to pass through *both* open states at the beginning and end of a burst. Openings other than the first and last in a burst will rarely get back to A_1 and 99.6% of the area is for the $\tau = 1.11$ ms component. The overall distribution of open times has 74% of the area for the $\tau = 1.11$ ms component (overall mean = 0.870 ms).

If the channel ever penetrates beyond A_1 to reach A_2 then it will probably oscillate several times between A_2 and B_3, so producing the component with many openings per burst.

A numerical example for **3**

By suitable choice of rate constants, the distribution of the number of openings per burst can be made the same as in the last example. Take $q_{14} = 3800\ \mathrm{s}^{-1}$, $q_{41} = 50\ \mathrm{s}^{-1}$, $q_{13} = 1200\ \mathrm{s}^{-1}$, $q_{31} = 5263.2\ \mathrm{s}^{-1}$, $q_{32} = 14736.8\ \mathrm{s}^{-1}$, $q_{23} = 1000\ \mathrm{s}^{-1}$. These rates give mean lifetimes for C_4, B_3, A_1, A_2 of 20 ms, 50 μs, 0.2 ms and 1 ms, exactly as in the last two examples.

The components of the distribution of the number of openings per burst will again have means $\mu_1 = 5$, $\mu_2 = 1$, i.e. $\lambda_1 = 0.8$, $\lambda_2 = 0$. In this case $\lambda_1 = \pi_{31}\pi_{13} + \pi_{32} = 1 - \pi_{31}\pi_{14}$. As in the previous example the component with one opening per burst ($\mu_2 = 1$) accounts for 70% of the area of the distribution ($a_2 = 0.7, a_1 = 1 - a_2 = \pi_{13}/\lambda_1 = 0.3$). Again 76% ($= 1 - \pi_{14}$) of *all* bursts have only one opening. Thus, in this particular case the distribution of the number of openings per burst is identical with that for **2**.

The distribution of open times is not greatly different from the last example either. The time constants in this case are exactly 1 ms and 0.2 ms, i.e. the mean lifetimes of the two open states (there is no direct communication between the two open states in this example, so each observed opening consists of a single sojourn in one or the other open state). The overall distribution of open times has 59.8% of the area for the 0.2 ms component (rather more than in the last case), and 40.2% for the 1 ms component. The correlation between open times is rather stronger than in the last case, $\rho(1) = 0.15$, though it decays at the same rate. The correlation (for one channel) between shut times is $\rho(1) = 0.20$, and between an open time and the following shut time, $\rho(1) = -0.23$. However, the burst structure is quite distinctive in the present case. Clearly bursts that have either one *or* two openings must consist entirely of sojourns in A_1 (mean length 0.2 ms), as must the first and last opening of *any* burst, so 100% of the area is associated with the $\tau = 0.2$ ms component for all such openings; they are simple exponential distributions (quite unlike the last example where the distributions went through a peak). Openings other than the first and last in the burst would not be so distinctive (92% of area for $\tau = 1$ ms, 8% for $\tau = 0.2$ ms).

In this case it is again obviously the $C_4 \to A_1 \to C_4$ transitions that mainly give rise to the isolated openings; if the channel penetrates as far as B_3 it is likely to oscillate several times between B_3 and A_2 before returning to the resting state, so giving rise to a component with many openings per burst.

A conjecture

It seems, from the discussion above, that a component with unit mean occurs when $R(\boldsymbol{X}_{\mathscr{A}\mathscr{A}}) > R(\boldsymbol{H}_{\mathscr{A}\mathscr{A}})$, and we suspect (but have not rigorously proved) that this is a general result. This happens when the connectivity between $\mathscr{A}$ and $\mathscr{F}$ $(= \mathscr{B} \cup \mathscr{C})$ is greater than the direct connectivity between $\mathscr{A}$ and $\mathscr{B}$ (see §1). For this to happen there must be a state in $\mathscr{A}$ that (*a*) is connected to $\mathscr{C}$ and (*b*) is *not* a direct gateway state (as defined in §1) between $\mathscr{A}$ and $\mathscr{B}$. For example, in scheme **3** of figure 2 state A_1 is connected to $\mathscr{C}$, but it is not a direct $\mathscr{A}$–$\mathscr{B}$ gateway state so the component with unit mean is seen, but in scheme **1** of figure 2 state A_1 is a direct $\mathscr{A}$–$\mathscr{B}$ gateway state and the component is not seen. Similarly, the unit mean component is seen in **23** (A_1 and A_2 are connected to $\mathscr{C}$ but A_1 is not a direct $\mathscr{A}$–$\mathscr{B}$ gateway state, though state A_2 is), but not in scheme **22** (states A_1 and A_3 are connected to $\mathscr{C}$, but both are direct $\mathscr{A}$–$\mathscr{B}$ gateway states).

Conversely, the area for a component with unit mean will be zero if either (*a*) there is no direct route from $\mathscr{A}$ to $\mathscr{C}$ ($\boldsymbol{Q}_{\mathscr{A}\mathscr{C}} = \boldsymbol{0}, D^{\mathrm{v}}(\mathscr{A},\mathscr{C}) = 0$) or (*b*) if there are direct routes from $\mathscr{A}$ to $\mathscr{C}$ but each such route has an $\mathscr{A}$ state as its direct gateway state *and* this state is also a direct gateway state between $\mathscr{A}$ states and $\mathscr{B}$ states (as well as between $\mathscr{A}$ states and $\mathscr{C}$ states). These conditions are exactly those which ensure that the rank of $\boldsymbol{X}_{\mathscr{A}\mathscr{A}}$ is the same as the rank of $\boldsymbol{H}_{\mathscr{A}\mathscr{A}}$, i.e. that (usually) the rank of $\boldsymbol{Q}_{\mathscr{A}\mathscr{F}}$ is the same as the rank of $\boldsymbol{Q}_{\mathscr{A}\mathscr{B}}$.

An example of the argument is provided by the scheme **21** in figure 2 in which $\boldsymbol{Q}_{\mathscr{A}\mathscr{F}}$ has the form

$$\boldsymbol{Q}_{\mathscr{A}\mathscr{F}} = [\boldsymbol{Q}_{\mathscr{A}\mathscr{B}} \quad \boldsymbol{Q}_{\mathscr{A}\mathscr{C}}] = \begin{array}{c} \begin{array}{cccccc} 4 & 5 & 6 & 7 & 8 & 9 \end{array} \\ \left[\begin{array}{ccc|ccc} \times & 0 & 0 & 0 & 0 & 0 \\ \times & 0 & \times & \times & 0 & \times \\ \times & 0 & 0 & 0 & 0 & 0 \end{array}\right] \end{array} \begin{array}{c} \\ 1 \\ 2 \\ 3. \end{array} \tag{7.10}$$

The partition of $\boldsymbol{Q}_{\mathscr{A}\mathscr{F}}$ into $\boldsymbol{Q}_{\mathscr{A}\mathscr{B}}$ and $\boldsymbol{A}_{\mathscr{A}\mathscr{C}}$ is shown by the dashed line; an $\times$ element represents any non-zero rate (i.e. a route exists) and a 0 element indicates that no route exists. The state numbers are shown in the margins. Note that the direct connectivity between $\mathscr{A}$ and $\mathscr{B}$ is $D^{\mathrm{v}}(\mathscr{A},\mathscr{B}) = 2$, the direct gateway states being A_2, B_4 and B_6. Deletion of two of these states (2, 4 or 4, 6) separates $\mathscr{A}$ and $\mathscr{B}$ completely, and deletion of the corresponding rows/columns in (7.10) leaves only zeros in $\boldsymbol{Q}_{\mathscr{A}\mathscr{B}}$. If we choose to delete row 2 and column 4 then we are also left with zeros in $\boldsymbol{Q}_{\mathscr{A}\mathscr{C}}$ (because state A_2 is the one direct $\mathscr{A}$–$\mathscr{C}$ gateway as well as being one of the direct $\mathscr{A}$–$\mathscr{B}$ gateway states). Thus there are only zeros left in the whole of $\boldsymbol{Q}_{\mathscr{A}\mathscr{F}}$; removal of states 2 and 4 (the two $\mathscr{A}$–$\mathscr{F}$ gateway states) also separates $\mathscr{A}$ and $\mathscr{F}$ completely. Thus $R(\boldsymbol{Q}_{\mathscr{A}\mathscr{B}}) = R(\boldsymbol{Q}_{\mathscr{A}\mathscr{F}}) = 2$ and the component with $\mu = 1$ in the distribution of the number of openings per burst will have zero area.

Discussion

Correlations

The existence of a 'gateway' through which the channel must pass during passage from 'open' to 'shut' (in the sense that deletion of a state disconnects open and shut states so the open–shut connectivity is 1) ensures that the durations of individual openings are uncorrelated; indeed it ensures that they are totally independent. Shut times are likewise uncorrelated, as are the durations of an opening and the duration of subsequent shuttings. The results of Fredkin *et al.* (1985) have been extended here to correlations between open times within a burst, and to correlations between burst lengths. Analogous rules concerning connectivity and gateway states are given for the latter cases.

Several groups have detected correlations in their data. Jackson *et al.* (1983) measured, essentially, an approximation to the correlation between the first and second apparent openings in a burst with acetylcholine (ACh) in cultured rat muscle. Labarca *et al.* (1985) reported correlation between apparent open times in reconstituted *Torpedo* ACh receptor channels. And Colquhoun & Sakmann (1985), working with frog end plate, found correlations between the lengths of apparent openings, and between the lengths of the first and second apparent opening in a burst. However, Colquhoun & Sakmann could detect no correlations between burst lengths; the comparison with the other work mentioned is made difficult because the resolution in Jackson *et al.* (1983) and Labarca *et al.* (1985) was 0.7–1 ms whereas in Colquhoun & Sakmann the resolution was 30–70 μs, so that what the former authors refer to as openings the latter would describe as bursts.

The qualitative existence of a correlation provides evidence for there being more than one pathway between the relevant subsets of states. In principle, the form of the decay of the correlation with increasing lag should tell us the number of such pathways, but problems caused by limited time resolution, and an unknown number of channels, have so far inhibited (wisely) any attempt to use correlations in such a quantitative way.

Negative correlations between the length of an opening and that of the following shut period have been detected by McManus *et al.* (1985). They did not calculate correlation coefficients, but rather the mean duration of all open intervals adjacent to shut intervals within a specified range of durations was plotted against the mean duration of the specified shut intervals; a clear relationship was found for both a chloride and a potassium channel. On the other hand, Colquhoun & Sakmann (1985) found nothing distinctive about the openings that border intermediate gaps within bursts. Clearly inspection of the actual structure of the sequence of open and shut times may give information about the way in which states are connected that is complementary to that found by measurement of correlation coefficients, and the other methods discussed here (as illustrated by the examples in §7, for instance).

The problem of the extent to which different reaction schemes can be distinguished from each other on the basis of experimental data is a complex one (even for ideal data). A start on the problem has been made by Fredkin *et al.* (1985) and

Fredkin & Rice (1986), but much remains to be done. The difficulties are well illustrated by the work of Horn & Vandenberg (1984) who used a full maximum likelihood analysis (which implicitly takes into account all information about correlations), and found that with real experimental data many reaction schemes were not clearly distinguishable.

Single channel events after a perturbation

There are many published results on, for example, the activation of sodium channels or of calcium channels following a voltage jump, or of ACh-activated channels after a concentration jump (see, for example, Chabala *et al.* 1985). However, we are not aware of any cases where differences in distributions for the first, second etc. opening have been investigated. The results given here show that substantial differences in the distributions (in the areas, rather than the time constants) may occur under certain circumstances. It is, however, shown here that no such differences are expected under conditions where events are uncorrelated.

Missing components in the distribution of the number of openings per burst

It is shown here that under certain circumstances the number of geometric components may, in principle, be less than the number of open states. The conditions under which a component with unit mean does, or does not, appear in this distribution are discussed; as with the appearance of correlations, these conditions depend on the nature of the connections between the various states of the system. This method was used by Colquhoun & Sakmann (1985) to rule out certain reaction schemes; in fact in their case the information inferred in this way was clearer than that inferred from the measurement of correlations.

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References

Aldrich, R. W., Corey, D. P. & Stevens, C. F. 1983 A reinterpretation of mammalian sodium channel gating based on single channel recording. *Nature, Lond.* **306**, 436–441.

Chabala, L. D., Gurney, A. M. & Lester, H. A. 1985 Photoactivation and dissociation of agonist molecules at the nicotinic acetylcholine receptor in voltage-clamped rat myoballs. *Biophys. J.* **48**, 241–246.

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

Colquhoun, D. & Hawkes, A. G. 1981 On the stochastic properties of single ion channels. *Proc. R. Soc. Lond.* B **211**, 205–235.

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

Colquhoun, D. & Hawkes, A. G. 1983 The principles of the stochastic interpretation of ion channel mechanisms. In *Single channel recording* (ed. B. Sakmann & E. Neher), pp. 135–175. New York: Plenum Press.

Colquhoun, D. & Sakmann, B. 1985 Fast events in single-channel currents activated by acetylcholine and its analogues at the frog muscle end-plate. *J. Physiol., Lond.* **369**, 501–557.

Colquhoun, D. & Sigworth, F. J. 1983 Fitting and statistical analysis of single channel records. In *Single channel recording* (ed. B. Sakmann & E. Neher), pp. 191–263. New York: Plenum Press.

Fredkin, D. R., Montal, M. & Rice, J. A. 1985 Identification of aggregated Markovian models: application to the nicotinic acetylcholine receptor. In *Proceedings of the Berkeley Conference in honor of Jerzy Neyman and Jack Kiefer* (ed. Lucien M. Le Carn & Richard A. Olshen), vol. 1, pp. 269–289. Wadsworth.

Fredkin, D. R. & Rice, J. A. 1986 On aggregated Markov processes. *J. appl. Prob.* **23**, 208–214.

Hawkes, A. G. & Colquhoun, D. 1987 (In preparation.)

Horn, R. & Vandenberg, C. A. 1984 Statistical properties of single sodium channels. *J. gen. Physiol.* **84**, 505–534.

Jackson, M. B., Wong, B. S., Morris, C. E., Lecar, H. & Christian, C. N. 1983 Successive openings of the same acetylcholine receptor channel are correlated in open time. *Biophys. J.* **42**, 109–114.

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

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

Mirsky, L. 1955 *An introduction to linear algebra.* Oxford University Press.

Tainiter, M. 1975 Statistical theory of connectivity. I. Basic definitions and properties. *Discrete Mathematics* **13**, 391–398.