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ON THE STOCHASTIC PROPERTIES OF BURSTS OF SINGLE ION CHANNEL OPENINGS AND OF CLUSTERS OF BURSTS

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CONTENTS

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Characteristics of observed bursts of single channel openings were derived recently for two particular ion channel mechanisms. In this paper these methods are generalized so that the observable characteristics of bursts can be calculated directly for any mechanism that has transition probabilities that are independent of time as long as the process is at equilibrium or is maintained in a steady state by an energy supply.

General expressions are given for the distributions of the open time, the number of openings per burst, the total open time per burst, the gaps within and between bursts, and so on.

With the aid of these general results a single computer program can be written that will provide numerical values for such distributions for *any* postulated mechanism, given only the transition rates between the various states.

The results are illustrated by a numerical example of a mechanism in which two agonist molecules can bind sequentially, and either singly or doubly occupied receptor ion channels may open.

The analogous theory is also given for the case where bursts of channel openings are grouped into clusters; many of the results bear a close analogy with those found for simple bursts.

Introduction

It is now possible to observe the currents that flow through several types of single ion channels in biological membranes (Neher & Sakmann 1976; Hamill *et al.* 1981). Channel types that have been studied include those that are opened by (*a*) acetylcholine-like agonists (Neher & Sakmann 1976; Sakmann *et al.* 1980), (*b*) glutamate (Patlak *et al.* 1979), (*c*) membrane depolarization (Conti & Neher 1980; Sigworth & Neher 1980) and (*d*) intracellular calcium ions (Marty 1981; Pallotta *et al.* 1981; Colquhoun *et al.* 1981).

In a number of cases it has been observed that two or more ion channel openings may occur in quick session (the *nachschlag* phenomenon), with the result that channel openings are grouped into more or less clearly defined bursts of closely spaced openings, separated by longer shut periods. Such bursts may be observed in the presence of agonist alone (Colquhoun & Sakmann 1981; Cull-Candy & Parker 1982), and also in the presence of an antagonist drug that can block the ion channels opened by the agonist (Neher & Steinbach 1978; Ogden *et al.* 1981). In at least one case it has been observed that, following several such bursts, a very long shut period occurs, so that the bursts of channel openings are clearly occurring in clusters (Sakmann *et al.* 1980). Some possible mechanisms that could account for such bursts, and clusters of bursts, will be mentioned later, in §1*b*, in the numerical example (§4), and in the Discussion.

The grouping of openings into bursts and clusters is, of course, merely a reflection of the existence of multiple shut (and possibly open) states, such that conventional macroscopic measurements of the total current flow, through a large number of ion channels, would result in relaxations that were not simple exponentials, but that could be described by a sum of several exponential terms, as discussed, for example, by Colquhoun & Hawkes (1977).

In a recent paper (Colquhoun & Hawkes 1981) we have provided a basis for predicting, given a postulated reaction mechanism, the behaviour of single ion channels. The problems of interpretation of experimental data stem largely from three sources, as follows.

(1) Although most plausible reaction mechanisms postulate several non-conducting (shut) states, and sometimes also more than one open state (all open states possibly having identical conductance), the actual observations generally show only whether the channel is conducting (open) or not (shut).

(2) In most cases it is not known how many individual ion channels may be contributing to the observed record. Therefore, if two successive openings do not originate from the same ion channel, the duration of the shut period between them cannot be interpreted simply. This is a great disadvantage, because, in so far as there are more shut states than open states, most of the information about mechanisms should come from measurements of shut time

durations rather than of open time durations. It is this fact that is one of the main incentives for attempting to group observed channel openings into bursts, because there is usually good reason to believe that at least all the openings in a given burst originate from the same ion channel, even though the next burst may originate from a different channel. Thus the length of the gaps *within* a burst can be interpreted simply, though the lengths of gaps *between* bursts may have no simple interpretation.

(3) In practice the frequency resolution of measurements will be limited; so it may be impossible to observe the shortest openings and gaps (see, for example, Colquhoun & Sakmann 1981). This limitation will affect the predicted form of the distributions. For example, if many short gaps remain undetected, the distribution of the open time will be seriously affected, because two or more openings in quick succession will be counted as a single opening. The modifications that must be made to the present results to allow for this problem are given by Hawkes & Colquhoun (1983).

Colquhoun & Hawkes (1981) considered these problems (except for the last), and gave a general method, applicable to any specified mechanism, for deriving the distribution of the length of time spent in any specified subset of states. They used these methods to derive the *observable* characteristics of bursts of channel openings (e.g. burst length), for two particular reaction mechanisms. These latter results were, however, not completely general, but were derived *ad hoc* for each particular mechanism; furthermore there are some mechanisms (e.g. those that involve more than one open state, and/or cyclic reactions) for which the appropriate extension of these methods is by no means obvious. It is our main purpose, in this paper, to present entirely general methods of deriving the distributions of *observable* characteristics of bursts of openings, such as the total burst length, the total open time per burst, the length of the kth shut period within a burst, and so on. These results will apply to any specified mechanism, regardless of the number of open states, or cyclic reactions (as along as the transition probabilities do not vary with time). We then show how this approach can be extended to deal with cases where clusters of bursts can be distinguished; the results show rather elegant analogies with those for the simpler burst analysis.

In practice there is, of course, no completely unambiguous way of telling whether any particular shut period is within a burst or not. This problem was considered by Colquhoun & Hawkes (1981), who described the use of Bayes's theorem to calculate the probabilities that a particular shut period is part of a burst; the problem is considered here (§ 1 *d*) from a rather different point of view.

Throughout this paper, the term *burst* (rather than *apparent burst* as in Colquhoun & Hawkes (1981)) will be used to describe the observable phenomenon, defined in figures 1 and 2.

1. Basic assumptions and definitions

(*a*) *Basic definitions*

As in our previous work (Colquhoun & Hawkes 1981) the behaviour of single ion channels is analysed in terms of a Markov process in continuous time. Thus it is assumed that the probability of transition from one state to another is a constant, independent of time. For unimolecular reactions this amounts to no more than the normal postulate of the law of mass action, that rate constants are indeed constant (as long as variables, such as temperature and membrane potential, to which the rate constant is sensitive are held constant). But for association reactions,

for which the transition probability involves ligand concentration as well as an association rate constant, the Markov assumption implies that ligand concentrations do not vary with time, and this we assume in all that follows.

Before discussing the methods to be used in detail, some examples will be given to illustrate the nature of the problem. These examples concern bursts of openings (which are analysed in detail later, in §3), but the same principles can be applied to the analysis of clusters of bursts (see §5). We define as k the number of kinetically distinguishable states in which the system can exist. To predict the characteristics of bursts of openings it is convenient to divide the k states in which the system can exist into three subsets, as follows.

(1) Subset $\mathscr{A}$ comprises the open states ($k_{\mathscr{A}}$ in number, say).

(2) Subset $\mathscr{B}$ comprises short-lived shut states ($k_{\mathscr{B}}$ in number), such that any two openings that are separated by a sojourn in $\mathscr{B}$ are counted as being part of the same burst of openings (and the intermediate sojourn in $\mathscr{B}$ is a 'gap within a burst').

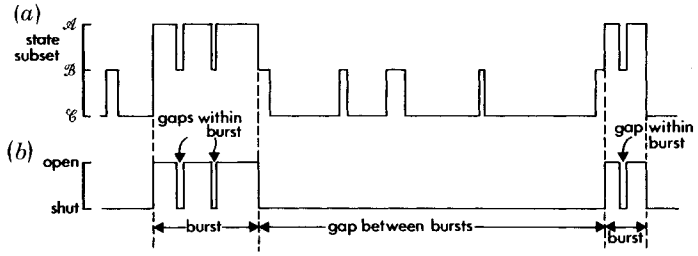

FIGURE 1. Diagrammatic representation of possible behaviour of a single ion channel, which has any mechanism that results in the occurrence of bursts of openings. The upper part shows the transitions of the system between the three subsets of states defined at the beginning of §1 ($\mathscr{A}$, open states; $\mathscr{B}$, gap within burst states; $\mathscr{C}$, gap between burst states). The lower part shows the corresponding appearance of the single channel current (when it is assumed, if there is more than one open state, that all have the same conductance). Two bursts are shown (the first with three openings and the second with two).

(3) Subset $\mathscr{C}$ comprises long-lived shut states ($k_{\mathscr{C}}$ in number) such that any entry into $\mathscr{C}$ results in a shut period so long that it is deemed to be part of a 'gap between bursts'.

(4) We also define subset $\mathscr{E} = \mathscr{A} \cup \mathscr{B}$, i.e. $\mathscr{E}$ contains all the states in $\mathscr{A}$, plus those in $\mathscr{B}$, $k_{\mathscr{E}} = k_{\mathscr{A}} + k_{\mathscr{B}}$ in number altogether.

(5) Similarly, we define $\mathscr{F} = \mathscr{B} \cup \mathscr{C}$, so that $\mathscr{F}$ contains all the states in $\mathscr{B}$ and $\mathscr{C}$, i.e. all the shut states, $k_{\mathscr{F}} = k_{\mathscr{B}} + k_{\mathscr{C}}$ in number.

An example of the possible behaviour of the system is shown in figure 1. This looks superficially like the illustrations in Colquhoun & Hawkes (1981), but in fact it is much more general; each of the three horizontal levels in figure 1*a* corresponds with a whole subset of states, for any mechanism (rather than with a single state, in a particular specified mechanism, as in figures 1 and 2 of Colquhoun & Hawkes (1981). The term *burst length*, as defined in figure 1 of the present paper, is used to describe the time from the start of the first opening to the end of the last opening in the burst, i.e. it is an experimentally observable quantity (the term 'apparent burst' used to describe the same thing in Colquhoun & Hawkes (1981) is no longer necessary).

First, we define a $k \times k$ matrix, $\boldsymbol{P}(t)$, with elements given by

$$P_{ij}(t) = P(\text{state } j \text{ at time } t \mid \text{state } i \text{ at time zero}), \tag{1.1}$$

where $P(\ )$ denotes the probability of the event in parentheses. It is then a standard result (see for example, Colquhoun & Hawkes 1977, 1981) that

$$\mathrm{d}\boldsymbol{P}(t)/\mathrm{d}t = \boldsymbol{P}(t)\,\boldsymbol{Q}, \tag{1.2}$$

where $\boldsymbol{Q}$ is a matrix with elements q_{ij} that are (for $i \neq j$) the usual law of mass action rate constants for transition from state i to state j. The diagonal elements ($i = j$) are constructed such that the row sums of $\boldsymbol{Q}$ are all zero, so that q_{ii} is minus the sum of all rate constants for leaving state i and is therefore negative. In stochastic terms, the mass action rate constants are defined, for $i \neq j$, as

$$q_{ij} = \lim_{\Delta t \to 0} [P(\text{in state } j \text{ at time } t+\Delta t \mid \text{in state } i \text{ at time } t)/\Delta t], \quad i \neq j. \tag{1.3}$$

Since $\boldsymbol{P}(0) = \boldsymbol{I}$, the unit matrix, the formal solution of (1.2) is

$$\boldsymbol{P}(t) = \mathrm{e}^{\boldsymbol{Q}t}, \tag{1.4}$$

where the exponential function is defined in terms of its expansion, namely $\mathrm{e}^{\boldsymbol{Q}t} = \boldsymbol{I} + \boldsymbol{Q}t + \boldsymbol{Q}^2t^2/2! + \ldots$.

If the system is in any state i, the probability that the next transition will be to state j, regardless of *when* the transition occurs, will be denoted π_{ij} and is given by

$$\pi_{ij} = q_{ij}/(-q_{ii}). \tag{1.5}$$

The sum of these, over all $j \neq i$, is simply unity (the transition must be to *somewhere*); this follows from the definition of q_{ii}.

The matrix, $\boldsymbol{Q}$, of transition rates can now be partitioned according to the subsets of states just defined; so it will have the form

$$\boldsymbol{Q} = \begin{bmatrix} \boldsymbol{Q}_{\mathscr{A}\mathscr{A}} & \boldsymbol{Q}_{\mathscr{A}\mathscr{B}} & \boldsymbol{Q}_{\mathscr{A}\mathscr{C}} \\ \boldsymbol{Q}_{\mathscr{B}\mathscr{A}} & \boldsymbol{Q}_{\mathscr{B}\mathscr{B}} & \boldsymbol{Q}_{\mathscr{B}\mathscr{C}} \\ \boldsymbol{Q}_{\mathscr{C}\mathscr{A}} & \boldsymbol{Q}_{\mathscr{C}\mathscr{B}} & \boldsymbol{Q}_{\mathscr{C}\mathscr{C}} \end{bmatrix}. \tag{1.6}$$

The submatrices here defined are the basis for all subsequent arguments. Some examples will now be discussed before proceeding with the theory.

(*b*) *Some possible mechanisms*

Three examples will be given of possible ion channel mechanisms. The first two are very simple because they contain only one state in each subset, and they do not need the full generality of the theory; they will be convenient for illustration of the results derived in §3, as the results are presented. The third mechanism (1.11) is more complex and needs (almost) the full generality of the theory to describe its behaviour, which will be illustrated by a numerical example in §4.

(i) *A simple agonist mechanism* (Castillo & Katz 1957)

$$\begin{array}{lccccc} & \mathrm{T} & \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} & \mathrm{AT} & \underset{\alpha}{\overset{\beta}{\rightleftharpoons}} & \mathrm{AR} \\ \text{state subset:} & \mathscr{C} & & \mathscr{B} & & \mathscr{A}, \end{array} \tag{1.7}$$

where T is the shut conformation of the receptor ion channel complex, R is open conformation, and A is the agonist molecule (concentration x_{A}). This is one of the mechanisms that was

discussed by Colquhoun & Hawkes (1981). The allocation of states to the subsets, $\mathscr{A}$, $\mathscr{B}$ and $\mathscr{C}$, in (1.7) is appropriate only if the agonist concentration is low, for the following reason. One would expect that openings could occur in bursts because, following any opening, there must necessarily be a sojourn in the shut but occupied state, AT (subset $\mathscr{B}$). If the channel then re-opened (rather than the agonist molecule dissociating) a second opening would appear shortly after the first; the burst would consist of oscillations between states AR and AT (i.e. between subsets $\mathscr{A}$ and $\mathscr{B}$ according to the general scheme defined above). However, if the channel, while in state AT, should next lose its agonist molecule, rather than re-opening, the resting state, T, would be reached. If the agonist concentration is low then state T will have a long lifetime; so the burst will come to an end. State T therefore constitutes the subset $\mathscr{C}$ in this case.

This example illustrates well the fact that the division of states into the subsets defined in §1*a* is not a characteristic of the reaction mechanism alone. This division will depend on the particular values of the rate constants and drug concentrations that are specified. In the above example, the lifetime of the resting state, T, is long enough for entry into it to produce a 'gap between bursts' only if the agonist concentration is low. At high agonist concentrations, openings would occur frequently and division of the record into bursts would not be obvious, according to the mechanism in (1.7). In fact Sakmann *et al.* (1980) did observe bursts of openings with high agonist concentration, but in their case the gaps between bursts were interpreted as involving entry into long-lived desensitized states which are not included in (1.7) (they would constitute subset $\mathscr{C}$ in the present notation). In this case the states AT and T would both be short-lived and would constitute the gaps within a burst (subset $\mathscr{B}$).

The matrix of transition rates for (1.7), partitioned as in (1.6), is

$$Q = \begin{array}{c} \\ \mathscr{A} \\ \mathscr{B} \\ \mathscr{C} \end{array} \begin{array}{c} \begin{array}{ccc} \mathscr{A} & \mathscr{B} & \mathscr{C} \end{array} \\ \left[\begin{array}{ccc} -\alpha & \alpha & 0 \\ \beta & -(\beta+k_{-1}) & k_{-1} \\ 0 & k_{+1}x_{\mathrm{A}} & -k_{+1}x_{\mathrm{A}} \end{array}\right] \end{array}. \tag{1.8}$$

In this case $k_{\mathscr{A}} = k_{\mathscr{B}} = k_{\mathscr{C}} = 1$ and all submatrices are scalar. Also $Q_{\mathscr{A}\mathscr{C}} = Q_{\mathscr{C}\mathscr{A}} = 0$ because the mechanism allows no direct transition between $\mathscr{A}$ and $\mathscr{C}$.

(ii) *A simple open ion channel block mechanism*

$$\begin{array}{lc} & \text{Shut} \underset{\alpha}{\overset{\beta'}{\rightleftharpoons}} \text{open} \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} \text{blocked} \\ \text{state subset:} & \mathscr{C} \qquad \mathscr{A} \qquad \mathscr{B}. \end{array} \tag{1.9}$$

In this mechanism, agonist binding is assumed to be fast compared with the open–shut conformation change, and β' is the effective rate constant for channel opening (see, for example Colquhoun & Hawkes 1977). The constants k_{+1} and k_{-1} represent the rate constants for association and dissociation of a blocking molecule (concentration x_{B}), which can combine with and block the open ion channel.

Oscillation between open and blocked states will result in the occurrence of openings in bursts, as long as the lifetime of the shut (as opposed to blocked) state is sufficiently long. This will be the case for an agonist-operated channel if the agonist concentration is sufficiently low.

In this case entry into the shut state (subset $\mathscr{C}$ in general) will result in a shut period much longer than that produced by a blockage; so the burst of openings will end.

The matrix of transition rates, partitioned as in (1.6) is

$$Q = \left[\begin{array}{c|c|c} -(\alpha + k_{+1}x_B) & k_{+1}x_B & \alpha \\ \hline k_{-1} & -k_{-1} & 0 \\ \hline \beta' & 0 & -\beta' \end{array}\right]. \tag{1.10}$$

Again $k_{\mathscr{A}} = k_{\mathscr{B}} = k_{\mathscr{C}} = 1$, but in this case $Q_{\mathscr{C}\mathscr{B}} = Q_{\mathscr{B}\mathscr{C}} = 0$ because the blocker is supposed to be able to react only with *open* channels.

(iii) *A more complex agonist mechanism*

There is a great deal of evidence that two molecules (at least) of acetylcholine are needed for efficient opening of the ion channels of the skeletal muscle endplate (see reviews, e.g Colquhoun 1979). There is also some reason to think that the channel may still be able to open, though with lower probability, when only one acetylcholine molecule is bound (Colquhoun 1973; Dionne *et al.* 1978; Colquhoun & Sakmann 1981). The simplest mechanism that fulfils these criteria is

$$\begin{array}{lccc} \text{state subset:} & \mathscr{C} & \mathrm{T} & \\ & & \Downarrow\uparrow & \\ & \mathscr{B} & \mathrm{AT} \rightleftharpoons \mathrm{AR} & \mathscr{A} \\ & & \Downarrow\uparrow \quad \Downarrow\uparrow & \\ & \mathscr{B} & \mathrm{A_2T} \rightleftharpoons \mathrm{A_2R} & \mathscr{A}. \end{array} \tag{1.11}$$

There are two open states ($k_{\mathscr{A}} = 2$), via either of which a particular opening may start, and end. Also there are two states in $\mathscr{B}$, via either of which a 'gap within a burst' may start, and end. To cope with this the full generality of the theory is needed, except that there is only one state in $\mathscr{C}$, and it cannot be reached directly from the open state; so $Q_{\mathscr{A}\mathscr{C}} = Q_{\mathscr{C}\mathscr{A}} = \mathbf{0}$ (this would not be so, for example, for the full Monod–Wyman–Changeux model, in which an unoccupied but open species, R, appears). This mechanism will be considered in detail later (§4).

(c) *Theoretical background*

(i) *General theory*

The crucial step for all that follows is the derivation of probabilities analogous with $p_{ij}(t)$, defined in (1.1), but such that the system remains within a specified subset of states, $\mathscr{A}$ say, *throughout* the time from 0 to t. These will be defined as

$$\begin{aligned} {}^{\mathscr{A}}p_{ij}(t) = P(&\text{system remains within } \mathscr{A} \text{ throughout time 0 to time } t, \\ &\text{and is in state } j \text{ at time } t \mid \text{in state } i \text{ at time 0}), \quad i,j \in \mathscr{A}. \end{aligned} \tag{1.12}$$

The addition rule of probability implies that

$$\begin{aligned} {}^{\mathscr{A}}p_{ij}(t+\Delta t) = \sum_{k\in\mathscr{A}} \{&P[\text{system in } \mathscr{A} \text{ throughout } (0, t) \text{ and state } k \text{ at } t \mid \text{state } i \text{ at time 0}] \\ &\times P(\text{state } j \text{ at } t+\Delta t \mid \text{state } k \text{ at } t)\}, \quad i,j \in \mathscr{A}. \end{aligned} \tag{1.13}$$

The first factor is, from (1.12), simply ${}^{\mathscr{A}}p_{ik}(t)$. The second factor, from (1.3), is (if $j \neq k$) $q_{kj}\Delta t + o(\Delta t)$, where $o(\Delta t)$ is a term that represents the possibility of there being more than one transition in Δt; it disappears when Δt is small enough (see, for example, Colquhoun 1971, Appendix 2). For the case $j = k$ the second factor represents the probability that the system does *not* move out of k during Δt, i.e. $1 - P(\text{leave } k) = 1 + q_{kk}\Delta t + o(\Delta t)$. Thus (1.13) can be written in matrix notation as

$$\boldsymbol{P}_{\mathscr{A}\mathscr{A}}(t+\Delta t) = \boldsymbol{P}_{\mathscr{A}\mathscr{A}}(t)\,[\boldsymbol{I} + \boldsymbol{Q}_{\mathscr{A}\mathscr{A}}\Delta t + o(\Delta t)], \tag{1.14}$$

where $\boldsymbol{P}_{\mathscr{A}\mathscr{A}}(t)$ is defined as the $(k_{\mathscr{A}} \times k_{\mathscr{A}})$ matrix with elements defined in (1.12), and $\boldsymbol{Q}_{\mathscr{A}\mathscr{A}}$ is the submatrix of $\boldsymbol{Q}$, defined in (1.6) and exemplified in (1.8) and (1.10). Note, however, that $\boldsymbol{P}_{\mathscr{A}\mathscr{A}}(t)$ is *not* simply a submatrix of $\boldsymbol{P}(t)$. If (1.14) is rearranged, and the limit $\Delta t \to 0$ is taken, we find

$$\mathrm{d}\boldsymbol{P}_{\mathscr{A}\mathscr{A}}(t)/\mathrm{d}t = \boldsymbol{P}_{\mathscr{A}\mathscr{A}}(t)\,\boldsymbol{Q}_{\mathscr{A}\mathscr{A}}, \tag{1.15}$$

the solution of which gives our required probabilities (1.12) in

$$\boldsymbol{P}_{\mathscr{A}\mathscr{A}}(t) = \exp(\boldsymbol{Q}_{\mathscr{A}\mathscr{A}}t). \tag{1.16}$$

Exactly analogous relations obviously hold for any other subset. The Laplace transform of $P_{\mathscr{A}\mathscr{A}}(t)$, which will frequently be needed, is, from (1.16),

$$\boldsymbol{P}^*_{\mathscr{A}\mathscr{A}}(s) = (s\boldsymbol{I} - \boldsymbol{Q}_{\mathscr{A}\mathscr{A}})^{-1}. \tag{1.17}$$

where s is the Laplace variable which has the dimensions of frequency.

We now wish, as in Colquhoun & Hawkes (1981), to define a density that describes the probability of staying within the subset of states $\mathscr{A}$ for a time t and then leaving $\mathscr{A}$ for a state outside $\mathscr{A}$ (in subset $\mathscr{B}$, say), i.e.

$$g_{ij}(t) = \lim_{\Delta t \to 0} [P(\text{stay in } \mathscr{A} \text{ from time 0 to time } t, \text{ and leave } \mathscr{A} \text{ for state } j \text{ between } t \text{ and } t+\Delta t \mid \text{in state } i \text{ at time 0})/\Delta t], \quad i \in \mathscr{A}, j \in \mathscr{B}. \tag{1.18}$$

It follows from (1.13) and (1.12) that when we add the probabilities for the routes from i to j via each possible intermediate state, r, we get

$$g_{ij}(t) = \sum_{r \in \mathscr{A}} {}^{\mathscr{A}}p_{ir}(t)\,q_{rj}, \quad i \in \mathscr{A}, j \in \mathscr{B}. \tag{1.19}$$

In matrix form, $g_{ij}(t)$ are, therefore, simply the elements of the $k_{\mathscr{A}} \times k_{\mathscr{B}}$ matrix

$$\boldsymbol{G}_{\mathscr{A}\mathscr{B}}(t) = \boldsymbol{P}_{\mathscr{A}\mathscr{A}}(t)\,\boldsymbol{Q}_{\mathscr{A}\mathscr{B}}. \tag{1.20}$$

The Laplace transform of this, from (1.17), is

$$\boldsymbol{G}^*_{\mathscr{A}\mathscr{B}}(s) = \boldsymbol{P}^*_{\mathscr{A}\mathscr{A}}(s)\,\boldsymbol{Q}_{\mathscr{A}\mathscr{B}} = (s\boldsymbol{I} - \boldsymbol{Q}_{\mathscr{A}\mathscr{A}})^{-1}\,\boldsymbol{Q}_{\mathscr{A}\mathscr{B}} \tag{1.21}$$

with elements $g^*_{ij}(s)$, say. It follows from (1.18) that the integral of $g_{ij}(t)$ gives

$$\int_0^t g_{ij}(t)\,\mathrm{d}t = P(\text{life within } \mathscr{A} \leqslant t \text{ and exits to } j \mid \text{starts in } i). \tag{1.22}$$

Note that $g_{ij}(t)$ is not, itself, a proper probability density function (p.d.f.) because it has not got unit area; to achieve unit area, it must be divided by the probability that, given the system starts in $i \in \mathscr{A}$, it eventually reaches $j \in \mathscr{B}$, namely, from (1.22),

$$\int_0^\infty g_{ij}(t)\,dt = P(\text{exits to } j \mid \text{starts in } i), \quad i \in \mathscr{A}, j \in \mathscr{B} \tag{1.23}$$

$$= g_{ij}^*(0). \tag{1.24}$$

The second form follows from the definition of the Laplace transform; the integral of $g_{ij}(t)$ in (1.23) can be found by putting $s = 0$ in its Laplace transform. Notice that the probabilities, $g_{ij}^*(0)$, given by (1.23), allow for the possibility of any number of transitions *within* $\mathscr{A}$ states (see (1.12)) before $\mathscr{A}$ is eventually left for $\mathscr{B}$. They give the transition probabilities from $\mathscr{A}$ states to $\mathscr{B}$ states. The matrix, $\mathbf{G}_{\mathscr{A}\mathscr{B}}^*(0)$, with elements that are the transition probabilities, $g_{ij}^*(0)$, will be denoted simply as $\mathbf{G}_{\mathscr{A}\mathscr{B}}$, throughout this paper, for brevity. Thus, from (1.21),

$$\mathbf{G}_{\mathscr{A}\mathscr{B}} \equiv \mathbf{G}_{\mathscr{A}\mathscr{B}}^*(0) = -\mathbf{Q}_{\mathscr{A}\mathscr{A}}^{-1}\mathbf{Q}_{\mathscr{A}\mathscr{B}}, \tag{1.25}$$

and similarly, for brevity,

$$g_{ij} \equiv g_{ij}^*(0). \tag{1.26}$$

Thus, by use of (1.25), $\mathbf{G}_{\mathscr{A}\mathscr{B}}$ can be calculated directly from the subsections of $\mathbf{Q}$ defined in (1.6). Exactly analogous relations hold, of course, for any other pair of subscripts.

If $\mathscr{A}$ contains only one state (as in (1.7) and (1.9)), then the transition probability g_{ij} is the same thing as π_{ij} defined in (1.5). This is because there is, in this case, no possibility of moving between different $\mathscr{A}$ states; the first transition that occurs must lead out of $\mathscr{A}$. More generally, the g_{ij} can be written as a suitable combination of the π values, based on listing all possible routes from i to j.

We can now define a probability density function for the lifetime of a sojourn in $\mathscr{A}$, given the starting and exit states, as

$$f_{ij}(t) = g_{ij}(t)/g_{ij}^*(0). \tag{1.27}$$

This corresponds to the distribution function

$$F_{ij}(t) = \int_0^t f_{ij}(t)\,dt = P(\text{life in } \mathscr{A} \leqslant t \mid \text{exits to } j \textit{ and} \text{ starts in } i), \quad i \in \mathscr{A}, j \in \mathscr{B}. \tag{1.28}$$

(ii) *Numerical evaluation of results*

To evaluate numerically the results derived below, we employ the spectral expansion of $\mathbf{Q}_{\mathscr{A}\mathscr{A}}$ (see, for example: Colquhoun & Hawkes 1977, equations (13)–(17); Bailey 1964, pp. 47, 80), namely

$$\mathbf{Q}_{\mathscr{A}\mathscr{A}} = \sum_{m=1}^{k_{\mathscr{A}}} \mathbf{A}_m \rho_m. \tag{1.29}$$

In this expression, the ρ_m are the eigenvalues of $\mathbf{Q}_{\mathscr{A}\mathscr{A}}$ (which we always assume to be distinct), and the matrices $\mathbf{A}_m$ can be calculated from the eigenvectors of $\mathbf{Q}_{\mathscr{A}\mathscr{A}}$. The eigenvalues and eigenvectors can be computed numerically (e.g. with Nottingham Algorithms Group subroutine F02AGF). Throughout this paper we shall, as in Colquhoun & Hawkes (1981), always use the minus eigenvalues of $\mathbf{Q}_{\mathscr{A}\mathscr{A}}$ (i.e. the eigenvalues of $-\mathbf{Q}_{\mathscr{A}\mathscr{A}}$), denoted $\lambda_m = -\rho_m$. These are positive rate constants. Once these have been found we can compute

$$\mathbf{P}_{\mathscr{A}\mathscr{A}}(t) = \sum_{m=1}^{k_{\mathscr{A}}} \mathbf{A}_m e^{-\lambda_m t}, \tag{1.30}$$

It will turn out that the p.d.fs, which are derived below, can all be expressed in the form

$$f(t) = \boldsymbol{b}\boldsymbol{P}_{\mathscr{A}\mathscr{A}}(t)\,\boldsymbol{c}, \tag{1.31}$$

where the factors that pre-multiply $\boldsymbol{P}_{\mathscr{A}\mathscr{A}}(t)$ have been reduced to a single $(1 \times k_{\mathscr{A}})$ vector, $\boldsymbol{b}$, and the post-multiplier is a $(k_{\mathscr{A}} \times 1)$ vector, $\boldsymbol{c}$. It follows that the p.d.f. (1.31) can be expressed in the entirely scalar form

$$f(t) = \sum_{m=1}^{k_{\mathscr{A}}} w_m \mathrm{e}^{-\lambda_m t}, \tag{1.32}$$

i.e. as the weighted sum of $k_{\mathscr{A}}$ exponential terms, in which the (scalar) coefficients are given by

$$w_m = \boldsymbol{b}\boldsymbol{A}_m\boldsymbol{c} = \sum_{i \in \mathscr{A}} \sum_{j \in \mathscr{A}} b_i a_{ijm} c_j, \tag{1.33}$$

where b_i, c_j and a_{ijm} are elements, respectively, of $\boldsymbol{b}$, $\boldsymbol{c}$ and $\boldsymbol{A}_m$.

(iii) *Another approach*

The doubly conditional distribution in (1.27) and (1.28) is that used by Colquhoun & Hawkes (1981). It may be noted at this point that in a mechanism in which a state $j \in \mathscr{B}$ is accessible from more than one $\mathscr{A}$ state these distributions cannot completely define the system. In this case one can define an alternative distribution, which defines the way in which a sojourn in $\mathscr{A}$ ends, by specifying the state in $\mathscr{A}$ *from* which exit from $\mathscr{A}$ occurs (rather than, as above, specifying the state in $\mathscr{B}$ *to* which exit from $\mathscr{A}$ occurs). Thus we define, by analogy with (1.18),

$$h_{ij}(t) = \lim_{\Delta t \to 0} [P(\text{stay in } \mathscr{A} \text{ from time 0 to time } t, \text{ and leave } \mathscr{A} \textit{ from} \text{ state } j \text{ between } t \text{ and } t+\Delta t \mid \text{in state } i \text{ at time 0})/\Delta t], \quad i, j \in \mathscr{A} \tag{1.34}$$

with Laplace transform denoted $h^*_{ij}(s)$. The analogue of (1.23) is

$$h^*_{ij}(0) = \int_0^\infty h_{ij}(t)\,\mathrm{d}t = P(\text{exits } \textit{from } j \mid \text{starts in } i), \quad i, j \in \mathscr{A}. \tag{1.35}$$

Arguments analogous to those used above show that the $k_{\mathscr{A}} \times k_{\mathscr{A}}$ matrix with elements $h_{ij}(t)$ is given by

$$\boldsymbol{H}_{\mathscr{A}\mathscr{A}}(t) = \boldsymbol{P}_{\mathscr{A}\mathscr{A}}(t)\,\boldsymbol{D}_{\mathscr{A}}, \tag{1.36}$$

where $\boldsymbol{P}_{\mathscr{A}\mathscr{A}}(t)$ was given in (1.7), and $\boldsymbol{D}_{\mathscr{A}}$ is a diagonal matrix with elements

$$d_{ii} = \sum_{r \in \mathscr{B}} q_{ir}, \quad i \in \mathscr{A}, \tag{1.37}$$

which measures the total transition rate away from a given state i, in $\mathscr{A}$, to any $\mathscr{B}$ state. Hence we can define a probability density function, $f'_{ij}(t)$, that is analogous with (1.27),

$$f'_{ij}(t) = h_{ij}(t)/h^*_{ij}(0) = {}^{\mathscr{A}}p_{ij}(t)/{}^{\mathscr{A}}p^*_{ij}(0), \quad i, j \in \mathscr{A}, \tag{1.38}$$

which corresponds to the distribution function (cf. (1.28))

$$\int_0^t f'_{ij}(t)\,\mathrm{d}t = P(\text{life in } \mathscr{A} \leqslant t \mid \text{exits } \textit{from } j \textit{ and} \text{ starts in } i), \quad i, j \in \mathscr{A}. \tag{1.39}$$

The second form of (1.38) follows because d_{jj} cancels (as long as it is not zero); the denominator ${}^{\mathscr{A}}p^*_{ij}(0)$ is, from (1.17), simply an element of $-\boldsymbol{Q}^{-1}_{\mathscr{A}\mathscr{A}}$.

(*d*) *Definition of bursts in practice*

The approach, through Bayes's theorem, given by Colquhoun & Hawkes (1981) requires more information about the system than we will commonly possess. Colquhoun & Sakmann (1981) defined a burst, empirically, as any series of openings separated by gaps that were all shorter than some specified duration, t_{crit} say. It is clear that, if t_{crit} were made short enough, every opening would be treated as a separate burst; on the other hand, if t_{crit} were made very long, the whole record would be counted as a single burst. If we suppose that the distribution of *all* shut periods in the record can be described by a p.d.f. that is the sum of several exponential terms,

$$f(t) = \sum w_m e^{-\lambda_m t}, \tag{1.40}$$

say, then clearly the above procedure, which defines any shut period longer than t_{crit} as a gap between bursts, will result (on average) in division of an observed record with $N+1$ openings (and N gaps) into a number of bursts given by

$$\begin{aligned} \text{number of interburst gaps} &= \text{number of bursts} - 1 \\ &= NP(\text{shut period} \geqslant t_{crit}) = N\int_{t_{crit}}^{\infty} \sum w_m e^{-\lambda_m t} dt \\ &= N \sum (w_m/\lambda_m) e^{-\lambda_m t_{crit}}. \end{aligned} \tag{1.41}$$

A plot of the number of bursts against t_{crit} will therefore be a sum of exponentials with the same rate constants as in the original distribution of all gaps (1.40), but with amplitudes that are the relative areas (w_m/λ_m) of the components of the original distribution. We wish to pick a value for t_{crit} in a region such that the number of bursts that it defines is insensitive to the exact value chosen. But (1.41) is a monotonically decreasing curve. Adequate separation into bursts will be achieved only if the time constants $(1/\lambda_m)$ in (1.41) are so well separated that, once the faster components have died away, the slow components remaining are so slow that, on the relevant time scale, the graph of (1.41) looks nearly horizontal. In this case the exact value chosen for t_{crit} is unimportant, within this near-horizontal range.

The definition of bursts will also be affected if some openings, and/or gaps, are too short to see; the modifications that must be made to the present results when the frequency resolution of measurements is limited are given by Hawkes & Colquhoun (1983).

2. Some standard methods

It may be helpful to those readers who are not familiar with stochastic processes if a brief outline is given here of some of the principles used to derive the results that follow.

(*a*) *Probability of occurrence of a specified sequence of events*

First consider the sort of problem in which we are interested only in the probability of a particular sequence of transitions, rather than in the time spent in each state. For example we might wish to know the probability that a burst contains a specified number of openings. The simplest example (e.g. (1.7) and (1.9)) is the case where $\mathscr{A}$ and $\mathscr{B}$ each contain only one state ($k_{\mathscr{A}} = k_{\mathscr{B}} = 1$), say state 1 is $\mathscr{A}$, state 2 is $\mathscr{B}$. What, then, is the probability, given that we start in $\mathscr{A}$ (state 1), of a transition to $\mathscr{B}$ (state 2), and then back again to $\mathscr{A}$? Clearly, from

(1.5), we just multiply the probabilities for these two transitions to give $\pi_{12}\pi_{21}$, or, from (1.23), (1.24) and (1.26),

$$g_{12}\,g_{21}. \tag{2.1}$$

Suppose now that (as in mechanism (1.11)) there are two states in $\mathscr{A}$ (states 1 and 2 say), and also two states in $\mathscr{B}$ (states 3 and 4 say). What now is the probability of a transition from $\mathscr{A}$ to $\mathscr{B}$ and back? To determine this we must specify not only that we start in $\mathscr{A}$, but also the relative probabilities that we start in state 1 or state 2; say these are ϕ_1 and ϕ_2 respectively ($\phi_1+\phi_2 = 1$ because we must start in *one* of the $\mathscr{A}$ states). Furthermore, because of the way the transition probabilities are defined (see (1.22) and subsequent text), they allow for the possibility of any number of transitions within $\mathscr{A}$, before $\mathscr{A}$ is left for $\mathscr{B}$. Consider first the case where we end up, after the sojourn in $\mathscr{B}$, back in state 1. If the sojourn in $\mathscr{A}$ starts in 1, then, after any number of transitions within $\mathscr{A}$, we reach state 3 in $\mathscr{B}$, and next, after any number of transitions within $\mathscr{B}$, we return to state 1 (in $\mathscr{A}$), then the probability for this sequence would be given by $g_{13}\,g_{31}$. Alternatively, if state 4 in $\mathscr{B}$ was reached rather than state 3, the probability for this route would be $g_{14}\,g_{41}$. Similarly, if the sojourn in $\mathscr{A}$ started in state 2, we would get probabilities $g_{23}\,g_{31}$ for returning to state 1 after reaching state 3 (in $\mathscr{B}$), and $g_{24}\,g_{41}$ for the route via state 4. If the probabilities for the various routes are assembled by use of the addition and multiplication rules of probability, we find that the probability of an $\mathscr{A}\to\mathscr{B}\to\mathscr{A}$ transition that *ends* in state 1 is

$$\phi_1(g_{13}\,g_{31}+g_{14}\,g_{41})+\phi_2(g_{23}\,g_{31}+g_{24}\,g_{41}). \tag{2.2}$$

This is simply the first element of the $1\times k_{\mathscr{A}}$ vector calculated as

$$\boldsymbol{\Phi}\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}}, \tag{2.3}$$

where, in this case ($k_{\mathscr{A}} = 2$, $k_{\mathscr{B}} = 2$),

$$\boldsymbol{\Phi} = (\phi_1 \;\; \phi_2),$$

$$\boldsymbol{G}_{\mathscr{A}\mathscr{B}} = \begin{bmatrix} g_{13} & g_{14} \\ g_{23} & g_{24} \end{bmatrix} \quad \boldsymbol{G}_{\mathscr{B}\mathscr{A}} = \begin{bmatrix} g_{31} & g_{32} \\ g_{41} & g_{42} \end{bmatrix}. \tag{2.4}$$

The second element of (2.3) is easily seen to be the equivalent of (2.2), but for the case where we end in state 2 rather than 1. The overall probability of the $\mathscr{A}\to\mathscr{B}\to\mathscr{A}$ transition is the sum of these two elements (if we end in $\mathscr{A}$ we must end in state 1 *or* state 2), namely

$$\boldsymbol{\Phi}\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}}\boldsymbol{u}_{\mathscr{A}}, \tag{2.5}$$

where $\boldsymbol{u}_{\mathscr{A}}$ is a unit vector ($k_{\mathscr{A}}\times 1$). Expressions such as (2.3) and (2.5) clearly hold however many states there are in $\mathscr{A}$ and $\mathscr{B}$, and such successive multiplication of the transition matrices for a specified route ($\mathscr{A}\to\mathscr{B}\to\mathscr{A}$ in this case) is the basis of most of the following results.

Frequently we wish to consider the probability of the occurrence of *either* 0, *or* 1, *or* 2... transitions from $\mathscr{A}$ to $\mathscr{B}$ and back. This will give rise to terms of the form $(\boldsymbol{I}-\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{-1}$, because (see (A 1.1))

$$\sum_{r=0}^{\infty}(\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^r = (\boldsymbol{I}-\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{-1}. \tag{2.6}$$

(*b*) *The duration of a specified sequence of events*

Next, the treatment above must be extended to allow us to find the distribution of the *time* taken for a specified set of transitions. The basic results that are needed are: (*a*) the p.d.f. of

the sum of any number of random intervals is the *convolution* of their individual p.d.fs; and (*b*) the Laplace transform of the required p.d.f. is therefore the *product* of the Laplace transforms of the individual p.d.fs (see, for example, Cox 1962, p. 10). Consider again the simplest case, with one state (state 1) in $\mathscr{A}$, and one (state 2) in $\mathscr{B}$. Suppose that again we are interested in an $\mathscr{A} \to \mathscr{B} \to \mathscr{A}$ (i.e. $1 \to 2 \to 1$) transition, but now we wish to know the p.d.f. of the total time spent in the initial sojourn in state 1, and in the sojourn in state 2. The Laplace transform of this p.d.f. can be found by multiplying the Laplace transforms of the appropriate densities, and will therefore be of the form

$$g_{12}^*(s)\, g_{21}^*(s) \tag{2.7}$$

(such terms may need to be divided by a suitable normalizing factor to ensure that the area under the final p.d.f. is unity). An alternative way to write this expression is, from (1.5) and (1.21),

$$f_1^*(s)\, \pi_{12} f_2^*(s)\, \pi_{21}, \tag{2.8}$$

where $f_1^*(s) = (-q_{11})/(s-q_{11})$ is the Laplace transform of the p.d.f. of a single sojourn in state 1, namely the simple exponential distribution $f_1(t) = (-q_{11})\, e^{q_{11}t}$. Where there is more than one state in $\mathscr{A}$ and/or $\mathscr{B}$, the appropriate combination of such terms is, just as above, most simply found by matrix multiplication (see (2.3) and (2.5)); so the form of the final p.d.f. will be (apart from a normalizing factor),

$$\boldsymbol{\Phi} \boldsymbol{G}_{\mathscr{A}\mathscr{B}}^*(s)\, \boldsymbol{G}_{\mathscr{B}\mathscr{A}}^*(s)\, \boldsymbol{u}_{\mathscr{A}}. \tag{2.9}$$

Furthermore, if we are interested only in the time spent, say, in $\mathscr{B}$, we can get the appropriate p.d.f. simply by putting $s = 0$ in the first factor, so that $\boldsymbol{G}_{\mathscr{A}\mathscr{B}}^*(s)$ is replaced (see (1.25)) simply by $\boldsymbol{G}_{\mathscr{A}\mathscr{B}}$, i.e. by the transition probabilities, regardless of time. Or, put another way, we integrate (see (1.23)) over all possible durations of the initial stay in $\mathscr{A}$. This sort of procedure is used repeatedly in the following sections.

3. The analysis of bursts

The analysis of bursts is based on the definition of the subsets of states, $\mathscr{A}$, $\mathscr{B}$ and $\mathscr{C}$, discussed at the beginning of §1, and on the consequent partition of the matrix of transition rates in (1.6).

The results that are given below are all valid whether or not the various open states in $\mathscr{A}$ are distinguishable by virtue of having different conductances. If the conductances did differ (as in Hamill & Sakmann (1981)), the current through the open channel could switch to different values during the open periods shown in figure 1*b*; but information about the system from this source is ignored in the present treatment. As will be mentioned below, many results simplify considerably in cases where there is only one sort of open state ($k_{\mathscr{A}} = 1$).

(*a*) *The start of a burst*

Before proceeding we need to know about the various ways in which a burst can start (see §2). Clearly (see figure 1) a burst must start in an open state (an $\mathscr{A}$ state), but, if there is more than one state in $\mathscr{A}$, we shall need the relative probabilities of the burst starting (i.e. the first opening of a burst starting) in each of these sorts of open state. These probabilities are denoted $\boldsymbol{\Phi}_{\mathrm{b}}$, a $(1 \times k_{\mathscr{A}})$ vector (the subscript b stands for *burst*). To calculate these probabilities we note that the period before the beginning of a burst is characterized by at least one sojourn in $\mathscr{C}$

(and sojourns in $\mathscr{C}$ occur only between bursts); so we can take as our starting point the fraction of channels that is in each of the $\mathscr{C}$ states at equilibrium. These are denoted $\boldsymbol{p}_{\mathscr{C}}(\infty)$, a $(1 \times k_{\mathscr{C}})$ vector. The number of transitions per unit time directly from $\mathscr{C}$ to $\mathscr{A}$ is $p_i(\infty)\, q_{ij}$, where $i \in \mathscr{C}$ and $j \in \mathscr{A}$. To this must be added the number of indirect transitions (via any of the $\mathscr{B}$ states) per unit time, i.e.

$$p_i(\infty) \sum_{k \in \mathscr{B}} q_{ik}\, g_{kj}, \quad i \in \mathscr{C}, j \in \mathscr{A}. \tag{3.1}$$

The result, in matrix notation, is thus

$$\boldsymbol{\Phi}_{\mathrm{b}} = \frac{\boldsymbol{p}_{\mathscr{C}}(\infty)\,(\boldsymbol{Q}_{\mathscr{C}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}} + \boldsymbol{Q}_{\mathscr{C}\mathscr{A}})}{\boldsymbol{p}_{\mathscr{C}}(\infty)\,(\boldsymbol{Q}_{\mathscr{C}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}} + \boldsymbol{Q}_{\mathscr{C}\mathscr{A}})\,\boldsymbol{u}_{\mathscr{A}}}. \tag{3.2}$$

The scalar denominator is merely the sum of the terms in the numerator, which is needed to normalize the probabilities so that their sum, $\boldsymbol{\Phi}_{\mathrm{b}}\boldsymbol{u}_{\mathscr{A}}$, is unity. Another justification of this result is given later (see (3.88) and (3.89)). The result in (3.2) is rather similar to that given by Colquhoun & Hawkes (1981, equation (1.27)), but it is not the same. The earlier version, which we shall now denote $\boldsymbol{\Phi}_{\mathrm{o}}$ (the subscript stands for *opening*), gave the probabilities of *any* opening (any sojourn in $\mathscr{A}$) starting in each of the $\mathscr{A}$ states (see (3.63) and Appendix 1). On the other hand, what we want now are the relative probabilities of the first opening in a burst starting in each of the $\mathscr{A}$ states, which is what is given by (3.2).

(i) *Some special cases*

There are a number of special cases in which the initial vector, $\boldsymbol{\Phi}_{\mathrm{b}}$, can be calculated with less knowledge of the $\mathscr{C}$ states than is suggested by (3.2). These may be valuable because, in cases where successive bursts are not known to come from the same ion channel, detailed information about $\mathscr{C}$ states may be difficult to obtain.

(1) Only one state in $\mathscr{A}$ ($k_{\mathscr{A}} = 1$). In this case $\boldsymbol{\Phi}_{\mathrm{b}}$ is scalar, and equal to unity.

(2) Only one state in $\mathscr{E} = \mathscr{A} \cup \mathscr{B}$ that can be reached directly from $\mathscr{C}$, and that state is in $\mathscr{A}$. Then $\boldsymbol{\Phi}_{\mathrm{b}} = (0\ 0 \ldots 1 \ldots 0)$, with the unity in the position corresponding to the accessible $\mathscr{A}$ state.

(3) Only one state in $\mathscr{E} = \mathscr{A} \cup \mathscr{B}$ that can be reached directly from $\mathscr{C}$, and that state is in $\mathscr{B}$. Then $\boldsymbol{\Phi}_{\mathrm{b}}$ is the row of $G_{\mathscr{B}\mathscr{A}}$ that corresponds with the accessible state, scaled to sum to unity.

(4) Only one state in $\mathscr{C}$ from which $\mathscr{E} = \mathscr{A} \cup \mathscr{B}$ can be reached. Then $\boldsymbol{\Phi}_{\mathrm{b}}$ is the row of $(\boldsymbol{Q}_{\mathscr{C}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}} + \boldsymbol{Q}_{\mathscr{C}\mathscr{A}})$ that corresponds to the $\mathscr{C}$ state in question, scaled to sum to unity.

(*b*) *The end of a burst*

A burst ends when an open period, in $\mathscr{A}$, leads to a sojourn in $\mathscr{C}$ before another opening occurs (see figure 1). The route from $\mathscr{A}$ to $\mathscr{C}$ may be via an intermediate sojourn in $\mathscr{B}$, or it may be direct. This argument allows us to assemble the probabilities for the end of burst, for each possible starting state in $\mathscr{A}$. These will be denoted by the $(k_{\mathscr{A}} \times 1)$ vector, $\boldsymbol{e}_{\mathrm{b}}$ (the subscript stands for burst), given by

$$\boldsymbol{e}_{\mathrm{b}} = (\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{C}} + \boldsymbol{G}_{\mathscr{A}\mathscr{C}})\,\boldsymbol{u}_{\mathscr{C}}. \tag{3.3}$$

The postmultiplication by the unit vector, $\boldsymbol{u}_{\mathscr{C}}$, sums the probabilities over all $\mathscr{C}$ states, because arrival in any $\mathscr{C}$ state ensures the end of the burst.

It will be useful to note (see Appendix 1, equation (A 1.8)) that

$$(\boldsymbol{I}-\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{-1}\boldsymbol{e}_{\mathrm{b}} = \boldsymbol{u}_{\mathscr{A}}, \tag{3.4}$$

i.e. $\boldsymbol{e}_{\mathrm{b}} = (\boldsymbol{I}-\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})\boldsymbol{u}_{\mathscr{A}}$. The meaning of this result is clear, especially in the scalar case; $\boldsymbol{e}_{\mathrm{b}}$ contains probabilities that, starting in $\mathscr{A}$, the burst then ends, and $\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}}$ contains probabilities of returning to $\mathscr{A}$ so that the burst continues; so $\boldsymbol{I}-\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}}$ represents the probability of *not* continuing, and therefore of ending.

(*c*) *The number of openings per burst*

Clearly there must be at least one opening if there is to be a burst at all, and the probability of this first opening starting in each of the $\mathscr{A}$ states is given by $\boldsymbol{\Phi}_{\mathrm{b}}$ (3.2). If the next transition leads back to $\mathscr{C}$, with probabilities $\boldsymbol{e}_{\mathrm{b}}$ (3.3), then the burst ends after one opening only; so the probability of having only one opening per burst is $P(1) = \boldsymbol{\Phi}_{\mathrm{b}}\boldsymbol{e}_{\mathrm{b}}$. If, however, the first opening is followed by transitions $\mathscr{A}\rightarrow\mathscr{B}$ and $\mathscr{B}\rightarrow\mathscr{A}$ (probabilities $\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}}$) there will be several openings before the burst ends (see §2). The probability of seeing r openings per burst is, therefore,

$$\begin{aligned} P(r) &= \boldsymbol{\Phi}_{\mathrm{b}}(\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{r-1}\boldsymbol{e}_{\mathrm{b}} \\ &= \boldsymbol{\Phi}_{\mathrm{b}}(\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{r-1}(\boldsymbol{I}-\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})\boldsymbol{u}_{\mathscr{A}}, \quad r = 1, 2, \ldots, \infty. \end{aligned} \tag{3.5}$$

It is clear, especially from the latter form, that this result is the matrix analogue of the simple geometric distribution (used, for example, by Colquhoun & Hawkes (1981, equation (2.3)). It follows from (3.5) that the probability of seeing *at least i* openings in a burst is

$$P(r \geqslant i) = \boldsymbol{\Phi}_{\mathrm{b}}(\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{i-1}\boldsymbol{u}_{\mathscr{A}}. \tag{3.6}$$

The mean number of openings per burst is (see (A 1.2), (A 1.24), (3.63) and (3.4))

$$\begin{aligned} E(r) = \sum_{r=1}^{\infty} rP(r) &= \boldsymbol{\Phi}_{\mathrm{b}}(\boldsymbol{I}-\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{-1}\boldsymbol{u}_{\mathscr{A}} \\ &= 1/\boldsymbol{\Phi}_{0}(\boldsymbol{I}-\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})\boldsymbol{u}_{\mathscr{A}} = 1/\boldsymbol{\Phi}_{0}\boldsymbol{e}_{\mathrm{b}}. \end{aligned} \tag{3.7}$$

Note that $1/E(r) = \boldsymbol{\Phi}_{0}\boldsymbol{e}_{\mathrm{b}}$ is the probability that a gap is between bursts rather than within a burst.

The number of gaps in a burst must always be one less than the number of openings (see figure 1); so the distribution of the gaps follows at once from these results.

The form of the distribution, (3.5), can be clarified if we express the $(k_{\mathscr{A}}\times k_{\mathscr{A}})$ matrix, $\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}}$, in the form of its spectral expansion (see (1.29)–(1.33)), namely

$$\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}} = \sum_{m=1}^{k_{\mathscr{A}}} \boldsymbol{A}_{m}\rho_{m}, \tag{3.8}$$

where the ρ_{m} are the eigenvalues of the matrix $\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}}$, which will be positive, and less than unity. The matrices $\boldsymbol{A}_{m}$ can be found from the eigenvectors of $\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}}$. This allows us to write the distribution, (3.5), in the form

$$P(r) = \boldsymbol{\Phi}_{\mathrm{b}}\sum(\boldsymbol{A}_{m}\rho_{m}^{r-1})\boldsymbol{e}_{\mathrm{b}} = \sum_{m=1}^{k_{\mathscr{A}}} w_{m}\rho_{m}^{r-1}. \tag{3.9}$$

Equation (3.9) has the form of the weighted sum of $k_{\mathscr{A}}$ geometric distributions, with (scalar) coefficients given (see (1.33)) by

$$w_m = \boldsymbol{\Phi}_{\mathrm{b}} \boldsymbol{A}_m \boldsymbol{e}_{\mathrm{b}}. \tag{3.10}$$

This result is not surprising in view of the fact that the geometric distribution is the discrete equivalent of the exponential distribution, and all the continuous distributions derived below are described by weighted sums of exponentials.

(i) *The case of a single open state*

In this case ($k_{\mathscr{A}} = 1$), the number of openings per burst should follow a simple geometric distribution (as shown for two particular mechanisms by Colquhoun & Hawkes (1981)). Also, both $\boldsymbol{\Phi}_{\mathrm{b}}$ and $\boldsymbol{u}_{\mathscr{A}}$ are just unity, and both $\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}}$ and $\boldsymbol{e}_{\mathrm{b}}$ are scalar. Thus (see (3.4))

$$P(r) = (\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{r-1}\boldsymbol{e}_{\mathrm{b}} = (\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{r-1}(1-\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}}) \tag{3.11}$$

with mean

$$E(r) = 1/(1-\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}}) = 1/\boldsymbol{e}_{\mathrm{b}}. \tag{3.12}$$

If there is also only one $\mathscr{B}$ state, then $\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}} = \pi_{12}\pi_{21}$.

(ii) *Distributions conditional on starting state*

The probability of r openings per burst, given that the burst starts in the ith open state, is simply the ith element of the $(k_{\mathscr{A}} \times 1)$ vector found by omitting the $\boldsymbol{\Phi}_{\mathrm{b}}$ from (3.5), i.e.

$$(\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{r-1}\boldsymbol{e}_{\mathrm{b}}, \tag{3.13}$$

with a corresponding vector of means

$$(\boldsymbol{I}-\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{-1}\boldsymbol{u}_{\mathscr{A}}. \tag{3.14}$$

These distributions will not be directly observable (unless the various open states are distinguishable by virtue of having different conductances). Nevertheless, they may be helpful in providing intuitive understanding of the behaviour of a mechanism, as is exemplified later (§4).

(iii) *Simple examples*

For the simple agonist mechanism, (1.7), $\boldsymbol{G}_{\mathscr{A}\mathscr{B}} = 1$ because $\mathscr{A}$ can go *only* to $\mathscr{B}$, and $\boldsymbol{G}_{\mathscr{B}\mathscr{A}}$, from (1.25), is $-\boldsymbol{Q}_{\mathscr{B}\mathscr{B}}^{-1}\boldsymbol{Q}_{\mathscr{B}\mathscr{A}} = \beta/(\beta+k_{-1})$. So, from (3.11) and (3.12), the number of openings per burst follows a geometric distribution with mean $1+\beta/k_{-1}$ (as found by a different route in Colquhoun & Hawkes (1981)).

For the channel block mechanism, (1.10), $\boldsymbol{G}_{\mathscr{A}\mathscr{B}} = k_{+1}x_{\mathrm{B}}/(\alpha+k_{+1}x_{\mathrm{B}})$ and $\boldsymbol{G}_{\mathscr{B}\mathscr{A}} = 1$; so, from (3.11) and (3.12), the number of openings per burst is geometrically distributed with mean $1+k_{+1}x_{\mathrm{B}}/\alpha$, i.e.

$$\text{mean number of gaps per burst} = k_{+1}x_{\mathrm{B}}/\alpha. \tag{3.15}$$

(*d*) *The burst length*

We are interested now in the length of time spent in the burst; so the appropriate densities, for various routes through the burst, must be convolved (as outlined in §2). The probabilities for various routes through the burst are as already found in (3.5). The burst may consist of any number (1, 2, ..., ∞) of openings and so we must sum over these possibilities, but with *s not*

set to zero for the periods in $\mathscr{A}$ and $\mathscr{B}$ that constitute the burst. The Laplace transform of the required p.d.f. is therefore

$$\left.\begin{aligned} f^*(s) &= \sum_{r=1}^{\infty} \boldsymbol{\Phi}_{\mathrm{b}}[\boldsymbol{G}^*_{\mathscr{A}\mathscr{B}}(s)\,\boldsymbol{G}^*_{\mathscr{B}\mathscr{A}}(s)]^{r-1}\,[\boldsymbol{G}^*_{\mathscr{A}\mathscr{B}}(s)\,\boldsymbol{G}_{\mathscr{B}\mathscr{C}} + \boldsymbol{G}^*_{\mathscr{A}\mathscr{C}}(s)]\,\boldsymbol{u}_{\mathscr{C}} \\ &= \boldsymbol{\Phi}_{\mathrm{b}}[\boldsymbol{I} - \boldsymbol{G}^*_{\mathscr{A}\mathscr{B}}(s)\,\boldsymbol{G}^*_{\mathscr{B}\mathscr{A}}(s)]^{-1}\,[\boldsymbol{G}^*_{\mathscr{A}\mathscr{B}}(s)\,\boldsymbol{G}_{\mathscr{B}\mathscr{C}} + \boldsymbol{G}^*_{\mathscr{A}\mathscr{C}}(s)]\,\boldsymbol{u}_{\mathscr{C}}. \end{aligned}\right\} \tag{3.16}$$

Note that the last term is the same as the 'end of burst' vector, $\boldsymbol{e}_{\mathrm{b}}$ (eq. (3.3)), except that the last period in $\mathscr{A}$ (but not that in $\mathscr{B}$, if any) is part of the burst, so that we do not set $s = 0$ in these terms. The transform is not directly invertible as it stands, but it is shown in appendix 1 (A 1.32) that inversion gives the p.d.f. as

$$f(t) = \boldsymbol{\Phi}_{\mathrm{b}}[\boldsymbol{P}_{\mathscr{E}\mathscr{E}}(t)]_{\mathscr{A}\mathscr{A}}\,(-\boldsymbol{Q}_{\mathscr{A}\mathscr{A}})\,\boldsymbol{e}_{\mathrm{b}}, \tag{3.17}$$

where $\boldsymbol{P}_{\mathscr{E}\mathscr{E}} = \exp(\boldsymbol{Q}_{\mathscr{E}\mathscr{E}}t)$, the subset $\mathscr{E}$ being defined as $\mathscr{A} \cup \mathscr{B}$ (§1a). This p.d.f. can be evaluated from the spectral expansion of $\boldsymbol{P}_{\mathscr{E}\mathscr{E}}(t)$, as described in (1.29)–(1.33). It will consist of the sum of $k_{\mathscr{E}} = k_{\mathscr{A}} + k_{\mathscr{B}}$ exponential terms with rate constants that are the eigenvalues of $-\boldsymbol{Q}_{\mathscr{E}\mathscr{E}}$, but the coefficients (1.33) will still be found by summing over $i \in \mathscr{A}$, $j \in \mathscr{A}$, because only the $\mathscr{A}$ subsection of $\boldsymbol{P}_{\mathscr{E}\mathscr{E}}(t)$ (see (A 1.31)) is used. The form of (3.17) is, intuitively, very reasonable; $[\boldsymbol{P}_{\mathscr{E}\mathscr{E}}(t)]_{\mathscr{A}\mathscr{A}}$ is the submatrix of $\boldsymbol{P}_{\mathscr{E}\mathscr{E}}(t)$, consisting of the rows and columns of $\boldsymbol{P}_{\mathscr{E}\mathscr{E}}(t)$ that correspond to the states in $\mathscr{A}$, and it gives the probability of remaining within the set of states $\mathscr{E} = \mathscr{A} \cup \mathscr{B}$ throughout the time from 0 to t, starting in $\mathscr{A}$ and being in $\mathscr{A}$ at time t. This is multiplied by

$$-\boldsymbol{Q}_{\mathscr{A}\mathscr{A}}\boldsymbol{e}_{\mathrm{b}} = (\boldsymbol{Q}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{C}} + \boldsymbol{Q}_{\mathscr{A}\mathscr{C}})\,\boldsymbol{u}_{\mathscr{C}}, \tag{3.18}$$

which is the transition rate out of $\mathscr{A}$ into $\mathscr{C}$ (possibly via $\mathscr{B}$). The appearance of $-\boldsymbol{Q}_{\mathscr{A}\mathscr{A}}$ in (3.17) bears a direct analogy with the simple exponential distribution (see (3.64)). The mean burst length is

$$m = \boldsymbol{\Phi}_{\mathrm{b}}(\boldsymbol{I} - \boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{-1}(-\boldsymbol{Q}_{\mathscr{A}\mathscr{A}}^{-1})\,(\boldsymbol{I} - \boldsymbol{Q}_{\mathscr{A}\mathscr{B}}\boldsymbol{Q}_{\mathscr{B}\mathscr{B}}^{-1}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})\,\boldsymbol{u}_{\mathscr{A}}. \tag{3.19}$$

This is, of course, the sum of the mean open and gap times per burst which are derived below in (3.26) and (3.41).

(i) *Distributions conditional on the starting state*

Again, the distributions of burst length, conditional on the burst starting in the ith open state, are the elements of the vector found simply by omitting the initial vector, $\boldsymbol{\Phi}_{\mathrm{b}}$, from (3.16) or (3.17). The corresponding vector of means is found by omitting $\boldsymbol{\Phi}_{\mathrm{b}}$ from (3.19).

(ii) *The case of a single open state*

In this case the mean burst length, from (3.19) and (3.4), reduces to

$$m = (1 - \boldsymbol{Q}_{\mathscr{A}\mathscr{B}}\boldsymbol{Q}_{\mathscr{B}\mathscr{B}}^{-1}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})/(\boldsymbol{Q}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{C}} + \boldsymbol{Q}_{\mathscr{A}\mathscr{C}})\,\boldsymbol{u}_{\mathscr{C}}. \tag{3.20}$$

For example, in the simple channel block mechanism, (1.9), for which $\boldsymbol{G}_{\mathscr{B}\mathscr{A}} = 1$ and $\boldsymbol{G}_{\mathscr{B}\mathscr{C}} = 0$, the mean burst length becomes

$$m = (1 + x_{\mathrm{B}}/K_{\mathrm{B}})/\alpha, \tag{3.21}$$

where $K_{\mathrm{B}} = k_{-1}/k_{+1}$ is the equilibrium constant for blocker binding. This result was given by Neher & Steinbach (1978).

(e) The total open time per burst

In this case the possible routes through the burst are just the same as in (3.5) or (3.16), but now we are interested only in the time spent in open ($\mathscr{A}$) states, not that spent in shut ($\mathscr{B}$) states. Therefore, for the reasons outlined in §2, we simply modify (3.16), by setting $s = 0$ in the term $\boldsymbol{G}^*_{\mathscr{B}\mathscr{A}}(s)$, because this term, which, from (1.21), is $\boldsymbol{P}^*_{\mathscr{B}\mathscr{B}}(s)\,\boldsymbol{Q}_{\mathscr{B}\mathscr{A}}$, represents time periods in $\mathscr{B}$. Laplace transform of the required p.d.f. is therefore

$$f^*(s) = \boldsymbol{\Phi}_{\mathrm{b}}[\boldsymbol{I} - \boldsymbol{G}^*_{\mathscr{A}\mathscr{B}}(s)\,\boldsymbol{G}_{\mathscr{B}\mathscr{A}}]^{-1}\,[\boldsymbol{G}^*_{\mathscr{A}\mathscr{B}}(s)\,\boldsymbol{G}_{\mathscr{B}\mathscr{C}} + \boldsymbol{G}^*_{\mathscr{A}\mathscr{C}}(s)]\,\boldsymbol{u}_{\mathscr{C}}. \tag{3.22}$$

This, as in the last section, cannot be inverted as it stands, but after rearrangement, with the aid of (1.17), (1.21) and (3.3), the required p.d.f. is found as

$$\begin{aligned} f(t) &= \boldsymbol{\Phi}_{\mathrm{b}} \exp(\boldsymbol{V}_{\mathscr{A}\mathscr{A}} t)\,(\boldsymbol{Q}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{C}} + \boldsymbol{Q}_{\mathscr{A}\mathscr{C}})\,\boldsymbol{u}_{\mathscr{C}} \\ &= \boldsymbol{\Phi}_{\mathrm{b}} \exp(\boldsymbol{V}_{\mathscr{A}\mathscr{A}} t)\,(-\boldsymbol{V}_{\mathscr{A}\mathscr{A}})\boldsymbol{u}_{\mathscr{A}}, \end{aligned} \tag{3.23}$$

where we define

$$\boldsymbol{V}_{\mathscr{A}\mathscr{A}} = \boldsymbol{Q}_{\mathscr{A}\mathscr{A}} + \boldsymbol{Q}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}}. \tag{3.24}$$

The second form of (3.23) is derived from (3.3) and (3.4), which show that

$$-\boldsymbol{V}_{\mathscr{A}\mathscr{A}}\boldsymbol{u}_{\mathscr{A}} = (\boldsymbol{Q}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{C}} + \boldsymbol{Q}_{\mathscr{A}\mathscr{C}})\,\boldsymbol{u}_{\mathscr{C}} = -\boldsymbol{Q}_{\mathscr{A}\mathscr{A}}\boldsymbol{e}_{\mathrm{b}}. \tag{3.25}$$

The p.d.f. in (3.23) is a direct matrix analogue of a simple exponential distribution (which has the form $\lambda \mathrm{e}^{-\lambda t}$), with a 'rate constant' of $-\boldsymbol{V}_{\mathscr{A}\mathscr{A}}$. The p.d.f. can be evaluated as the weighted sum of $k_{\mathscr{A}}$ exponential terms, with rate constants that are the eigenvalues of $-\boldsymbol{V}_{\mathscr{A}\mathscr{A}}$, and coefficients that can be found by direct analogy with (1.29)–(1.33).

The mean of this distribution, i.e. the mean total open time per burst, is

$$\begin{aligned} m &= \boldsymbol{\Phi}_{\mathrm{b}}(\boldsymbol{I} - \boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{-1}\,(-\boldsymbol{Q}^{-1}_{\mathscr{A}\mathscr{A}})\,\boldsymbol{u}_{\mathscr{A}} \\ &= \boldsymbol{\Phi}_{\mathrm{b}}(-\boldsymbol{V}^{-1}_{\mathscr{A}\mathscr{A}})\,\boldsymbol{u}_{\mathscr{A}}. \end{aligned} \tag{3.26}$$

The first expression for the mean is seen to be the mean open time (from (3.62)) multiplied by the mean number of openings per burst (from (3.7)). The second expression is a direct matrix analogue of the mean of a simple exponential (which is λ^{-1}).

The distributions conditional on which state the burst starts in can be found, as in previous sections, by omitting $\boldsymbol{\Phi}_{\mathrm{b}}$ from (3.23) and (3.26).

This case (and the next) are rather unusual in that they involve eigenvalues of a matrix ($-\boldsymbol{V}_{\mathscr{A}\mathscr{A}}$ here) that is not a simple submatrix of $\boldsymbol{Q}$. However the form of the result can be seen to be intuitively reasonable by consideration of the following example.

(i) *A simple example*

Consider the channel blocking mechanism defined in (1.9) and (1.10). In this case the total rate of leaving the open state is $-\boldsymbol{Q}_{\mathscr{A}\mathscr{A}} = \alpha + k_{+1}x_{\mathrm{B}}$, the reciprocal of which is the mean lifetime of a single opening. In other words (apart from remainder terms, see (1.13) and (1.14)) the probability of leaving $\mathscr{A}$ (for either $\mathscr{B}$ *or* $\mathscr{C}$) during Δt is $-\boldsymbol{Q}_{\mathscr{A}\mathscr{A}}\Delta t = (\alpha + k_{+1}x_{\mathrm{B}})\,\Delta t$. Imagine, now, that a clock is started at the beginning of the first opening in a burst, and that the clock is stopped while the channel is blocked, and started again when the channel re-opens. We are interested in the time shown on the clock when the channel finally shuts (as opposed to blocks) because this will be the total open time per burst. As far as the time shown on the

clock is concerned, the transition rate for leaving $\mathscr{A}$ is not $-\boldsymbol{Q}_{\mathscr{A}\mathscr{A}}$; to allow for the fact that the channel may block, rather than shut, during Δt we must subtract, from $-\boldsymbol{Q}_{\mathscr{A}\mathscr{A}}$, the rate of transition to the blocked state, $\boldsymbol{Q}_{\mathscr{A}\mathscr{B}}$ (i.e. $k_{+1}x_{\mathrm{B}}$ in this example), times the probability, $\boldsymbol{G}_{\mathscr{B}\mathscr{A}}$, that, having blocked, the channel eventually re-opens, so that the burst continues (in this example $\boldsymbol{G}_{\mathscr{B}\mathscr{A}} = 1$ because there is no other way out of the blocked state except for re-opening). Thus, instead of $-\boldsymbol{Q}_{\mathscr{A}\mathscr{A}}$, we have

$$(-\boldsymbol{Q}_{\mathscr{A}\mathscr{A}}) - \boldsymbol{Q}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}} = -\boldsymbol{V}_{\mathscr{A}\mathscr{A}}. \tag{3.27}$$

This justifies the form of $\boldsymbol{V}_{\mathscr{A}\mathscr{A}}$ (3.24). In the present example

$$-\boldsymbol{V}_{\mathscr{A}\mathscr{A}} = (\alpha + k_{+1}x_{\mathrm{B}}) - k_{+1}x_{\mathrm{B}} = \alpha. \tag{3.28}$$

Therefore, since $\boldsymbol{\Phi}_{\mathrm{b}} = 1$ and $\boldsymbol{u}_{\mathscr{A}} = 1$, the distribution of the total open time per burst is, from (3.23),

$$f(t) = \alpha \mathrm{e}^{-\alpha t}, \tag{3.29}$$

i.e. a simple exponential distribution with mean $1/\alpha$. This is of course exactly the same as the distribution of the open lifetime in the absence of the channel blocking drug, as was first noted by Neher & Steinbach (1978). During the time that the clock is running the probability of the channel shutting (as opposed to blocking) during Δt is $\alpha \Delta t$; this ensures that the time shown on the clock when shutting eventually occurs, which will be the total open time per burst, will be exponentially distributed with mean $1/\alpha$, as found in (3.29).

(ii) *The case of a single open state*

In this case $\boldsymbol{\Phi}_{\mathrm{b}} = 1$, and $\boldsymbol{Q}_{\mathscr{A}\mathscr{A}} = q_{11}$ is scalar, as is $-\boldsymbol{V}_{\mathscr{A}\mathscr{A}} = -q_{11} - \boldsymbol{Q}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}}$. Therefore the distribution of the total open time per burst becomes the simple exponential p.d.f.,

$$f(t) = -\boldsymbol{V}_{\mathscr{A}\mathscr{A}} \exp(\boldsymbol{V}_{\mathscr{A}\mathscr{A}} t) = -q_{11}\, \boldsymbol{e}_{\mathrm{b}} \exp(q_{11}\, \boldsymbol{e}_{\mathrm{b}} t), \tag{3.30}$$

with mean

$$\begin{aligned} m &= -1/\boldsymbol{V}_{\mathscr{A}\mathscr{A}} = -1/q_{11}\boldsymbol{e}_{\mathrm{b}} = (\text{mean life in } \mathscr{A})/\boldsymbol{e}_{\mathrm{b}} \\ &= 1/(\boldsymbol{Q}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{C}} + \boldsymbol{Q}_{\mathscr{A}\mathscr{C}})\, \boldsymbol{u}_{\mathscr{C}}. \end{aligned} \tag{3.31}$$

Some interesting conclusions follow directly from these results. For example, notice that $\boldsymbol{Q}_{\mathscr{A}\mathscr{C}}\boldsymbol{u}_{\mathscr{C}}$ is the total transition rate from $\mathscr{A}$ to $\mathscr{C}$; if there is only one route from $\mathscr{A}$ to $\mathscr{C}$, with rate constant α say, then $\boldsymbol{Q}_{\mathscr{A}\mathscr{C}}\boldsymbol{u}_{\mathscr{C}} = \alpha$. The mean open time per burst would then become

$$m = 1/(\alpha + \boldsymbol{Q}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{C}}\boldsymbol{u}_{\mathscr{C}}), \tag{3.32}$$

i.e. it is less than $1/\alpha$ to an extent that depends on the rate of shutting via $\mathscr{B}$. If shutting via $\mathscr{B}$ is impossible (as, for example, in some channel block mechanisms for which $\boldsymbol{G}_{\mathscr{B}\mathscr{C}} = 0$) then, regardless of how many states there are in $\mathscr{B}$ and $\mathscr{C}$, the mean open time per burst becomes simply

$$m = 1/\alpha. \tag{3.33}$$

This result is invoked by Neher (1983), and by Ogden *et al.* (1983).

(*f*) *The total shut time per burst*

The distribution of the total length of all the gaps between openings, within a burst (see figure 1), can be found by modification of (3.16) in a manner exactly analogous to that used to find the total open time per burst, in (3.22). This time we are not interested in the open

times (times spent in $\mathscr{A}$); so we set $s = 0$ in the terms that represent periods in $\mathscr{A}$ (i.e. in $\boldsymbol{G}_{\mathscr{A}\mathscr{B}}$ and $\boldsymbol{G}_{\mathscr{A}\mathscr{C}}$), according to the principles that were outlined in §2. The Laplace transform of the required p.d.f. is therefore

$$f^*(s) = \boldsymbol{\Phi}_{\mathrm{b}}[\boldsymbol{I} - \boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}^*_{\mathscr{B}\mathscr{A}}(s)]^{-1}\boldsymbol{e}_{\mathrm{b}}, \tag{3.34}$$

where $\boldsymbol{e}_{\mathrm{b}}$ was defined in (3.3). After some rearrangement, this can be written as

$$f^*(s) = \boldsymbol{\Phi}_{\mathrm{b}}[\boldsymbol{I} + \boldsymbol{G}_{\mathscr{A}\mathscr{B}}(s\boldsymbol{I} - \boldsymbol{W}_{\mathscr{B}\mathscr{B}})^{-1}\boldsymbol{Q}_{\mathscr{B}\mathscr{A}}]\boldsymbol{e}_{\mathrm{b}}, \tag{3.35}$$

where we define

$$\boldsymbol{W}_{\mathscr{B}\mathscr{B}} = \boldsymbol{Q}_{\mathscr{B}\mathscr{B}} + \boldsymbol{Q}_{\mathscr{B}\mathscr{A}}\boldsymbol{G}_{\mathscr{A}\mathscr{B}}. \tag{3.36}$$

Inversion of (3.35) gives the p.d.f. as

$$f(t) = \boldsymbol{\Phi}_{\mathrm{b}}\boldsymbol{e}_{\mathrm{b}}\delta(t) + \boldsymbol{\Phi}_{\mathrm{b}}\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\exp(\boldsymbol{W}_{\mathscr{B}\mathscr{B}}t)\,\boldsymbol{Q}_{\mathscr{B}\mathscr{A}}\boldsymbol{e}_{\mathrm{b}} \tag{3.37}$$

$$= \boldsymbol{\Phi}_{\mathrm{b}}\boldsymbol{e}_{\mathrm{b}}\delta(t) + \boldsymbol{\Phi}_{\mathrm{b}}\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\exp(\boldsymbol{W}_{\mathscr{B}\mathscr{B}}t)\,(-\boldsymbol{W}_{\mathscr{B}\mathscr{B}})\,\boldsymbol{G}_{\mathscr{B}\mathscr{A}}\boldsymbol{u}_{\mathscr{A}}, \tag{3.38}$$

where $\delta(t)$ is a delta function (i.e. a spike with unit area, but with infinite height and infinitesimal width) at time zero. This first term simply represents all those bursts that have only one opening, and which therefore have identical (zero) total gap time per burst; from (3.5) the probability of a burst having one opening (and therefore no gaps) is $P(1) = \boldsymbol{\Phi}_{\mathrm{b}}\boldsymbol{e}_{\mathrm{b}}$, which, from (3.37), is exactly the area under the spike part of the p.d.f. It may be convenient in practice to look at the distribution of total gap time per burst for only those bursts that have at least one gap (i.e. at least two openings). This is given simply by the second term in (3.37) or (3.38), divided by the probability that there are at least two openings, i.e. from (3.6),

$$P(r \geqslant 2) = 1 - \boldsymbol{\Phi}_{\mathrm{b}}\boldsymbol{e}_{\mathrm{b}} = \boldsymbol{\Phi}_{\mathrm{b}}\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}}\boldsymbol{u}_{\mathscr{A}}. \tag{3.39}$$

This gives

$$f'(t) = \boldsymbol{\Phi}_{\mathrm{b}}\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\exp(\boldsymbol{W}_{\mathscr{B}\mathscr{B}}t)\,\boldsymbol{Q}_{\mathscr{B}\mathscr{A}}\boldsymbol{e}_{\mathrm{b}}/P(r \geqslant 2). \tag{3.40}$$

This p.d.f., and that in (3.38), can be expressed as the weighted sum of $k_{\mathscr{B}}$ (the number of states in $\mathscr{B}$) exponential terms, with rate constants that are the eigenvalues of $-\boldsymbol{W}_{\mathscr{B}\mathscr{B}}$, and coefficients that can be evaluated by direct analogy with (1.29)–(1.33).

The mean shut time per burst, from (3.37), is

$$\begin{aligned} m &= \boldsymbol{\Phi}_{\mathrm{b}}\boldsymbol{G}_{\mathscr{A}\mathscr{B}}(-\boldsymbol{W}^{-1}_{\mathscr{B}\mathscr{B}})\,\boldsymbol{G}_{\mathscr{B}\mathscr{A}}\boldsymbol{u}_{\mathscr{A}} \\ &= \boldsymbol{\Phi}_{\mathrm{b}}(\boldsymbol{I} - \boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{-1}\boldsymbol{G}_{\mathscr{A}\mathscr{B}}(-\boldsymbol{Q}^{-1}_{\mathscr{B}\mathscr{B}})\,\boldsymbol{G}_{\mathscr{B}\mathscr{A}}\boldsymbol{u}_{\mathscr{A}}. \end{aligned} \tag{3.41}$$

This mean includes all those bursts with zero shut time (only one opening). When these are excluded, as in (3.40), the mean becomes

$$m' = m/P(r \geqslant 2). \tag{3.42}$$

It may be noted that all these results for the total shut time per burst bear a close analogy to those just found for the total open time per burst, and they can be rationalized in an exactly analogous way.

The distributions conditional on the particular open state (the ith say) in which a burst starts are given simply by omitting $\boldsymbol{\Phi}_{\mathrm{b}}$ from (3.38) and (3.41); in the case of the conditional results (3.40) and (3.42) the ith distribution needs to be normalized, not by $P(r \geqslant 2)$, but by $1 - e_i$, where e_i is the ith element of $\boldsymbol{e}_{\mathrm{b}}$.

(i) *The case of a single state in $\mathscr{B}$*

Simplified results can be found if the gaps within a burst consist of a sojourn in a single state only. In this case $\boldsymbol{W}_{\mathscr{B}\mathscr{B}}$ is scalar, and the p.d.f. in (3.38) and (3.40) become simple exponential distributions. The mean shut time per burst becomes, from (3.39) and (3.41),

$$m = \boldsymbol{\Phi}_{\mathrm{b}} \boldsymbol{G}_{\mathscr{A}\mathscr{B}} \boldsymbol{G}_{\mathscr{B}\mathscr{A}} \boldsymbol{u}_{\mathscr{A}} / (-\boldsymbol{W}_{\mathscr{B}\mathscr{B}}) = P(r \geqslant 2)/(-\boldsymbol{W}_{\mathscr{B}\mathscr{B}}) \tag{3.43}$$

and the corresponding mean for bursts that have at least one gap is therefore, from (3.42) and (A 1.9),

$$\begin{aligned} m' = 1/(-\boldsymbol{W}_{\mathscr{B}\mathscr{B}}) &= (\text{mean life in } \mathscr{B})/(\boldsymbol{G}_{\mathscr{B}\mathscr{A}} \boldsymbol{G}_{\mathscr{A}\mathscr{C}} + \boldsymbol{G}_{\mathscr{B}\mathscr{C}}) \boldsymbol{u}_{\mathscr{C}} \\ &= 1/(\boldsymbol{Q}_{\mathscr{B}\mathscr{C}} + \boldsymbol{Q}_{\mathscr{B}\mathscr{A}} \boldsymbol{G}_{\mathscr{A}\mathscr{C}}) \boldsymbol{u}_{\mathscr{C}}. \end{aligned} \tag{3.44}$$

This last result shows, for example, that if there is only one direct route from $\mathscr{B}$ to $\mathscr{C}$, with rate constant k_{-1} say, then the total direct transition rate from $\mathscr{B}$ to $\mathscr{C}$, $\boldsymbol{Q}_{\mathscr{B}\mathscr{C}} \boldsymbol{u}_{\mathscr{C}}$, is simply k_{-1}, and m' is therefore less than $1/k_{-1}$ to an extent that depends on the transition rate for the indirect route from $\mathscr{B}$ to $\mathscr{C}$ via $\mathscr{A}$. If the latter route is impossible (as, for example, in the simple agonist mechanism discussed below), then the mean gap time per burst for bursts with at least one gap, m', becomes simply $1/k_{-1}$.

(ii) *Simple examples*

For the simple agonist mechanism (1.7) and (1.8), $\boldsymbol{Q}_{\mathscr{A}\mathscr{C}} = 0$, $\boldsymbol{G}_{\mathscr{A}\mathscr{B}} = 1$, and $\boldsymbol{G}_{\mathscr{B}\mathscr{A}} = \beta/(\beta + k_{-1})$; so, from (3.39), $P(r \geqslant 2) = \beta(\beta + k_{-1})$, and from (3.44), $-\boldsymbol{W}_{\mathscr{B}\mathscr{B}} = \boldsymbol{Q}_{\mathscr{B}\mathscr{C}} = k_{-1}$. The mean shut time per burst is therefore

$$m = \beta/k_{-1}(\beta + k_{-1}), \tag{3.45}$$

as given by Colquhoun & Hawkes (1981). But the mean shut time per burst for bursts that have at least one gap is, from (3.44), simply

$$m' = 1/k_{-1}. \tag{3.46}$$

For the simple channel block mechanism, (1.9) and (1.10), we have $\boldsymbol{G}_{\mathscr{B}\mathscr{A}} = 1$, and $\boldsymbol{G}_{\mathscr{A}\mathscr{B}} = k_{+1}x_{\mathrm{B}}/(\alpha + k_{+1}x_{\mathrm{B}}) = P(r \geqslant 2)$, from (3.39). Also $-\boldsymbol{W}_{\mathscr{B}\mathscr{B}} = \alpha k_{-1}/(\alpha + k_{+1}x_{\mathrm{B}})$; so the mean shut time per burst becomes

$$m = (x_{\mathrm{B}}/K_{\mathrm{B}})/\alpha, \tag{3.47}$$

where $K_{\mathrm{B}} = k_{-1}/k_{+1}$ is the equilibrium constant for antagonist binding. Similarly

$$m' = m + (1/k_{-1}). \tag{3.48}$$

(g) *The length of individual openings*

When we consider the distribution of the length of a single opening, a complication, which is unexpected at first sight, is encountered if there is more than one open ($\mathscr{A}$) state and more than one state in $\mathscr{B}$ (gaps within burst). We have obtained, in (3.2), the probabilities that a burst starts in each of the $\mathscr{A}$ states, i.e. that the first opening of a burst starts in the specified state. But, in general, the probabilities that the second opening of the burst starts in each open state may be different; they will depend on how the previous opening ended. For example, in the mechanism discussed in detail later (see §4), the probability that the second opening starts

in A_2R rather than in AR will clearly be greater if the first opening ended with an $A_2R \rightarrow A_2T$ transition than if it ended with an $AR \rightarrow AT$ transition. Thus we expect that, in general, the distribution of the length of the kth opening in a burst that has r openings will depend on both k and r. These distributions will be discussed first, then their equivalent when we average over different burst lengths and position within the burst.

(i) *Distribution of the kth opening in a burst with r openings*

We proceed as in previous sections, according to the principles outlined in §2. For all openings but the last, the following argument can be used. The kth opening, the duration of which we want to investigate (hence the $G^*_{\mathscr{A}\mathscr{B}}(s)$ term), must be preceded by $k-1$ openings, and $k-1$ gaps, the durations of which are irrelevant; so we set $s = 0$, giving the $(\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{k-1}$ term. The kth opening will then be followed by a further $r-k$ gaps and $r-k-1$ openings, the durations of which are again irrelevant; this takes us up to the start of the last opening, which, since, it is the last, gives rise to the end vector, $\boldsymbol{e}_b$, defined in (3.3). Thus the Laplace transform of the required p.d.f. is

$$f^*(s) = \boldsymbol{\Phi}_b(\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{k-1}\boldsymbol{G}^*_{\mathscr{A}\mathscr{B}}(s)\,\boldsymbol{G}_{\mathscr{B}\mathscr{A}}(\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{r-k-1}\boldsymbol{e}_b/P(r), \quad k < r, \tag{3.49}$$

where $P(r)$ is the probability that a burst contains r openings, given in (3.5). This scalar denominator is necessary so that the total area under the p.d.f., $f^*(0)$, is unity, as is seen by setting $s = 0$ in (3.49), and using (3.5). The last opening in a burst, unlike earlier openings, may terminate by a transition directly to $\mathscr{C}$; so its distribution can be found from

$$f^*(s) = \boldsymbol{\Phi}_b(\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{k-1}[\boldsymbol{G}^*_{\mathscr{A}\mathscr{B}}(s)\,\boldsymbol{G}_{\mathscr{B}\mathscr{C}} + \boldsymbol{G}^*_{\mathscr{A}\mathscr{C}}(s)]\boldsymbol{u}_{\mathscr{C}}/P(r), \quad k = r. \tag{3.50}$$

Although the separate forms, (3.49) and (3.50), just given are those with the most obvious derivation, both can be encompassed in the single equation

$$f^*(s) = \boldsymbol{\Phi}_b(\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{k-1}\boldsymbol{P}^*_{\mathscr{A}\mathscr{A}}(s)\,(-\boldsymbol{Q}_{\mathscr{A}\mathscr{A}})\,(\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{r-k}\boldsymbol{e}_b/P(r), \quad k \leqslant r. \tag{3.51}$$

It may be noted that the first part of (3.49)–(3.51), $\boldsymbol{\Phi}_b(\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{k-1}$, is a vector that gives the relative probabilities of the kth opening in the burst starting in each $\mathscr{A}$ state, just as $\boldsymbol{\Phi}_b$ (to which it reduces for $k = 1$) does for the first opening.

Inversion of (3.51) gives the p.d.f. as

$$f(t) = \boldsymbol{\Phi}_b(\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{k-1}\boldsymbol{P}_{\mathscr{A}\mathscr{A}}(t)\,(-\boldsymbol{Q}_{\mathscr{A}\mathscr{A}})\,(\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{r-k}\boldsymbol{e}_b/P(r), \quad k \leqslant r. \tag{3.52}$$

This can be expressed as the sum of $k_{\mathscr{A}}$ exponential terms, with rate constants that are the eigenvalues of $-\boldsymbol{Q}_{\mathscr{A}\mathscr{A}}$, and coefficients that can be found by direct analogy with (1.29)–(1.33).

The mean open time is

$$m = \boldsymbol{\Phi}_b(\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{k-1}(-\boldsymbol{Q}^{-1}_{\mathscr{A}\mathscr{A}})\,(\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{r-k}\boldsymbol{e}_b/P(r), \quad k \leqslant r. \tag{3.53}$$

These distributions are symmetrical in time for any mechanism that obeys the principle of microscopic reversibility (see below). This time symmetry means that the first and last openings in a burst have the same distribution (and hence the same mean length), the second and the next-to-last openings have the same distribution (which is, in general, different from that of the first and last openings), and so on. This time-reversible behaviour is the stochastic consequence (see Kelly 1979) of the principle of microscopic reversibility, or detailed balance

(Onsager 1931; Tolman 1938; Denbigh 1951). This principle states that at equilibrium the transition rates for every individual reaction step are the same in both directions, i.e.

$$p_i(\infty)\, q_{ij} = p_j(\infty)\, q_{ji}. \tag{3.54}$$

This result implies that, for any cyclic mechanism (see §4, for example), the product of the rate constants going one way round the cycle is equal to the product going the other way round; if states 1, 2, ..., n are arranged in a cycle so state 1 is connected to state n, then

$$q_{12}q_{23}\cdots q_{n1} = q_{1n}\cdots q_{32}q_{21}. \tag{3.55}$$

This principle must be obeyed by any reaction mechanism that can attain true thermodynamic equilibrium. It should, however, be stressed that all of the results derived in this paper (except for the time-reversible behaviour mentioned above) assume only that the system is in a steady state, not necessarily that it is in true equilibrium. The results are equally valid for irreversible processes, as long as the process is maintained in a steady state by means of a supply of energy.

If there is only one open state all of these complications disappear: $(\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})$ and $\boldsymbol{e}_{\mathrm{b}}$ are scalar and so factor out; all openings have the same simple exponential distribution given below in (3.66), regardless of k and r.

Various other distributions can be found from these results. For example the distribution of the length of the last opening in a burst would result from setting $k = r$ in (3.52), multiplying by $P(r)$, and summing over all numbers of openings ($r = 1, 2, \ldots, \infty$), which gives

$$f(t) = \boldsymbol{\Phi}_{\mathrm{b}}(\boldsymbol{I} - \boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{-1}\boldsymbol{P}_{\mathscr{A}\mathscr{A}}(t)\,(-\boldsymbol{Q}_{\mathscr{A}\mathscr{A}})\,\boldsymbol{e}_{\mathrm{b}}.$$

(ii) *Distribution of the kth opening in a burst (regardless of r)*

It may also be of interest to predict the distribution of the kth opening in a burst, regardless of how many openings there are in the burst. The distribution in (3.51) was conditional on the burst having r openings; we therefore, from the rules for conditional probability, multiply it by $P(r)$ and sum over all possible values of r, i.e. $r = k, k+1, \ldots, \infty$ (k can obviously not be greater than r, so $r \geqslant k$). The result (see also (3.4)), when properly normalized, is

$$f^*(s) = \boldsymbol{\Phi}_{\mathrm{b}}(\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{k-1}\boldsymbol{P}^*_{\mathscr{A}\mathscr{A}}(s)\,(-\boldsymbol{Q}_{\mathscr{A}\mathscr{A}})\,\boldsymbol{u}_{\mathscr{A}}/\boldsymbol{\Phi}_{\mathrm{b}}(\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{k-1}\boldsymbol{u}_{\mathscr{A}}. \tag{3.56}$$

The denominator in this is, from (3.6), the probability that a burst will contain at least k openings. Inversion of the Laplace transform gives the required p.d.f. as

$$f(t) = \boldsymbol{\Phi}_{\mathrm{b}}(\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{k-1}\boldsymbol{P}_{\mathscr{A}\mathscr{A}}(t)\,(-\boldsymbol{Q}_{\mathscr{A}\mathscr{A}})\,\boldsymbol{u}_{\mathscr{A}}/\boldsymbol{\Phi}_{\mathrm{b}}(\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{k-1}\boldsymbol{u}_{\mathscr{A}}. \tag{3.57}$$

This p.d.f. can be expressed as the sum of $k_{\mathscr{A}}$ exponential terms, with rate constants that are the eigenvalues of $-\boldsymbol{Q}_{\mathscr{A}\mathscr{A}}$, and coefficients that can be found as in (1.29)–(1.33). The mean open time for the kth opening in a burst is

$$m = \boldsymbol{\Phi}_{\mathrm{b}}(\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{k-1}(-\boldsymbol{Q}^{-1}_{\mathscr{A}\mathscr{A}})\,\boldsymbol{u}_{\mathscr{A}}/\boldsymbol{\Phi}_{\mathrm{b}}(\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{k-1}\boldsymbol{u}_{\mathscr{A}}. \tag{3.58}$$

When there is only one open state, again the complications disappear and the above results reduce to the simple overall exponential distribution given in (3.66), regardless of k.

(iii) *Overall distribution of the length of an opening*

To obtain the overall distribution of all open times, regardless of any possible grouping into bursts, we can simply multiply (3.57) by the probability of getting at least k openings per burst,

and sum over all possible values of k (i.e. $k = 1, \ldots, \infty$). Alternatively we can start directly from the p.d.f. in (3.52) that depends on both k and r, $f_{k,r}(t)$ say, and calculate

$$\sum_{r=1}^{\infty}\sum_{k=1}^{r} f_{k,r}(t)\,P(r) = \sum_{k=1}^{\infty}\sum_{r=k}^{\infty} f_{k,r}(t)\,P(r). \tag{3.59}$$

Either route, when properly normalized, gives

$$f(t) = \boldsymbol{\Phi}_{\mathrm{b}}(\boldsymbol{I}-\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{-1}\boldsymbol{P}_{\mathscr{A}\mathscr{A}}(t)\,(-\boldsymbol{Q}_{\mathscr{A}\mathscr{A}})\,\boldsymbol{u}_{\mathscr{A}}/E(r), \tag{3.60}$$

where $E(r)$ is the mean number of openings per burst, given in (3.7), i.e.

$$E(r) = \boldsymbol{\Phi}_{\mathrm{b}}(\boldsymbol{I}-\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{-1}\boldsymbol{u}_{\mathscr{A}}. \tag{3.61}$$

The overall mean open time is, therefore,

$$m = \boldsymbol{\Phi}_{\mathrm{b}}(\boldsymbol{I}-\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{-1}(-\boldsymbol{Q}_{\mathscr{A}\mathscr{A}}^{-1})\,\boldsymbol{u}_{\mathscr{A}}/E(r). \tag{3.62}$$

This is simply the mean open time per burst, from (3.26), divided by the mean number of openings per burst.

Alternative results for the distribution of open times were given by Colquhoun & Hawkes (1977), who presented a method for calculating the p.d.f. of the lifetime in any specified subset of states. In that work, grouping into bursts was not considered and so it was natural to use an initial vector, $\boldsymbol{\Phi}_{\mathrm{o}}$ (see Appendix 1), that gave the probability of each opening starting in a specified open state; all shut states were grouped into one subset, which was denoted $\mathscr{T}$, but which we here denote as $\mathscr{F} = \mathscr{B} \cup \mathscr{C}$. In the present notation, equation (65) of Colquhoun & Hawkes (1977) becomes

$$\boldsymbol{\Phi}_{\mathrm{o}} = \boldsymbol{p}_{\mathscr{F}}(\infty)\,\boldsymbol{Q}_{\mathscr{F}\mathscr{A}}/\boldsymbol{p}_{\mathscr{F}}(\infty)\,\boldsymbol{Q}_{\mathscr{F}\mathscr{A}}\boldsymbol{u}_{\mathscr{A}}. \tag{3.63}$$

The relation between $\boldsymbol{\Phi}_{\mathrm{o}}$ and $\boldsymbol{\Phi}_{\mathrm{b}}$ given in appendix 1, (A 1.24), shows that the overall distribution of open times, (3.60), can be written as

$$f(t) = \boldsymbol{\Phi}_{\mathrm{o}}\boldsymbol{P}_{\mathscr{A}\mathscr{A}}(t)\,(-\boldsymbol{Q}_{\mathscr{A}\mathscr{A}})\,\boldsymbol{u}_{\mathscr{A}} = \boldsymbol{\Phi}_{\mathrm{o}}\exp(\boldsymbol{Q}_{\mathscr{A}\mathscr{A}}t)\,(-\boldsymbol{Q}_{\mathscr{A}\mathscr{A}})\,\boldsymbol{u}_{\mathscr{A}}, \tag{3.64}$$

which is a direct matrix analogue of a simple exponential distribution, and is identical with equation (66) of Colquhoun & Hawkes (1977). Similarly the overall mean open time, (3.62), can be written

$$m = \boldsymbol{\Phi}_{\mathrm{o}}(-\boldsymbol{Q}_{\mathscr{A}\mathscr{A}}^{-1})\,\boldsymbol{u}_{\mathscr{A}}. \tag{3.65}$$

For a single open state, (3.64) and (3.65) become simply

$$f(t) = -q_{11}\mathrm{e}^{q_{11}t}, \tag{3.66}$$

$$m = 1/(-q_{11}), \tag{3.67}$$

an exponential distribution with mean, for example, of $1/\alpha$ for the simple agonist mechanism (1.7), and $1/(\alpha + k_{+1}x_{\mathrm{B}})$ for the channel block mechanism, (1.9).

(*h*) *Shut periods (gaps) within bursts*

As with open times (see §3*g*), the distributions of the first, second, ..., gaps within a burst may not be identical, if there is more than one open ($\mathscr{A}$) state, and more than one gap ($\mathscr{B}$) state. Again this happens because the way in which a gap starts may depend on how the preceding opening ended, which, in turn, may depend on how the previous gap ended.

(i) *Distribution of the kth gap in a burst with r openings*

The Laplace transform of the required p.d.f. is found by exactly the same sort of reasoning as for the corresponding open time distribution (3.49). In this case, however, we are interested in the time spent in the kth gap ($\mathscr{B}$ states; see figure 1), rather than the time spent in the kth opening; so the central term becomes (see §3) $\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}^{*}_{\mathscr{B}\mathscr{A}}(s)$ rather than $\boldsymbol{G}^{*}_{\mathscr{A}\mathscr{B}}(s)\,\boldsymbol{G}_{\mathscr{B}\mathscr{A}}$. Thus we obtain

$$f^{*}(s) = \boldsymbol{\Phi}_{\mathrm{b}}(\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{k-1}\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}^{*}_{\mathscr{B}\mathscr{A}}(s)\,(\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{r-k-1}\boldsymbol{e}_{\mathrm{b}}/P(r), \quad k = 1, \ldots, r-1, \quad (3.68)$$

where $P(r)$ is the probability that a burst has r openings (and therefore $r-1$ gaps) from (3.5). There is no need for a separate expression for the last gap because the last gap ends in exactly the same way as any other, with a transition to one of the open states. As in (3.51) inversion is straightforward, and gives (see (1.21)) the p.d.f. of the duration of the kth gap in a burst with r openings as

$$f(t) = \boldsymbol{\Phi}_{\mathrm{b}}(\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{k-1}\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{P}_{\mathscr{B}\mathscr{B}}(t)\,\boldsymbol{Q}_{\mathscr{B}\mathscr{A}}(\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{r-k-1}\boldsymbol{e}_{\mathrm{b}}/P(r), \quad k = 1, \ldots, r-1. \quad (3.69)$$

This, like the other distributions of gaps within bursts, can be expressed as the sum of $k_{\mathscr{B}}$ exponential terms, with rate constants that are the eigenvalues of $-\boldsymbol{Q}_{\mathscr{B}\mathscr{B}}$, and coefficients that can be found as in (1.29)–(1.33). The mean length of the kth gap in a burst with r openings is

$$m = \boldsymbol{\Phi}_{\mathrm{b}}(\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{k-1}\boldsymbol{G}_{\mathscr{A}\mathscr{B}}(-\boldsymbol{Q}^{-1}_{\mathscr{B}\mathscr{B}})\,\boldsymbol{G}_{\mathscr{B}\mathscr{A}}(\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{r-k-1}\boldsymbol{e}_{\mathrm{b}}/P(r). \quad (3.70)$$

These results show the same symmetry in time as the openings do, and for the same reason (described following (3.53)).

If there is only one state in $\mathscr{B}$, then $\boldsymbol{\Phi}_{\mathrm{b}}(\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{k-1}\boldsymbol{G}_{\mathscr{A}\mathscr{B}}$ and $\boldsymbol{Q}_{\mathscr{B}\mathscr{A}}(\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{r-k-1}\boldsymbol{e}_{\mathrm{b}}$ are both scalar and so factor out of (3.69) and (3.70), leaving the simple exponential distribution of gap times given in (3.80) and (3.81). If there is only one open ($\mathscr{A}$) state then $\boldsymbol{\Phi}_{\mathrm{b}}$, $\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}}$ and $\boldsymbol{e}_{\mathrm{b}}$ are scalar and therefore factor out, giving the overall intraburst gap distribution specified in (3.78) and (3.79). In both of these special cases the distribution become the same for all gaps within a burst, regardless of k and r.

(ii) *Distribution of the kth gap in a burst (regardless of r)*

The distribution of the duration of the kth gap within a burst, regardless of how many openings the burst may contain, can be found by similar reasoning to that used for (3.56). The result, which is conditional on r (3.68), is multiplied by $P(r)$ and summed over all possible r values, i.e. $r = k+1, \ldots, \infty$ (because a burst with r openings has $r-1$ gaps, and so $k \leqslant r-1$, i.e. $r \geqslant k+1$). The Laplace transform of the p.d.f. is therefore

$$f^{*}(s) = \boldsymbol{\Phi}_{\mathrm{b}}(\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{k-1}\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}^{*}_{\mathscr{B}\mathscr{A}}(s)\boldsymbol{u}_{\mathscr{A}}/\boldsymbol{\Phi}_{\mathrm{b}}(\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{k}\boldsymbol{u}_{\mathscr{A}}. \quad (3.71)$$

This follows from (3.4). The denominator is chosen to ensure that the area under the p.d.f., $f^{*}(0)$ (see (1.23)), is unity; it is, as would be expected, the probability that a burst will contain at least k gaps (from (3.6)). Inversion of (3.71) gives the p.d.f. as

$$f(t) = \boldsymbol{\Phi}_{\mathrm{b}}(\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{k-1}\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{P}_{\mathscr{B}\mathscr{B}}(t)\,\boldsymbol{Q}_{\mathscr{B}\mathscr{A}}\boldsymbol{u}_{\mathscr{A}}/\boldsymbol{\Phi}_{\mathrm{b}}(\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{k}\boldsymbol{u}_{\mathscr{A}}. \quad (3.72)$$

The mean duration of the kth gap is given by

$$m = \boldsymbol{\Phi}_{\mathrm{b}}(\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{k-1}\boldsymbol{G}_{\mathscr{A}\mathscr{B}}(-\boldsymbol{Q}_{\mathscr{B}\mathscr{B}}^{-1})\,\boldsymbol{G}_{\mathscr{B}\mathscr{A}}\boldsymbol{u}_{\mathscr{A}}/\boldsymbol{\Phi}_{\mathrm{b}}(\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{k}\boldsymbol{u}_{\mathscr{A}}. \tag{3.73}$$

If there is only one state in either $\mathscr{A}$ or $\mathscr{B}$, the dependence on k disappears and (3.72) reduces to the corresponding overall results, (3.78) and (3.80) respectively.

(iii) *Overall distribution of gaps within bursts*

To obtain the overall distribution of all gaps within bursts we can multiply (3.71) by the probability that a burst contains at least k gaps, and sum over all possible k ($k = 1, \ldots, \infty$). Equivalently we can start from the p.d.f. that depends on both k and r, $f_{k,r}(t)$, from (3.69) and calculate

$$\sum_{r=2}^{\infty}\sum_{k=1}^{r-1} f_{k,r}(t)\,P(r) = \sum_{k=1}^{\infty}\sum_{r=k+1}^{\infty} f_{k,r}(t)\,P(r). \tag{3.74}$$

Either approach gives, when properly normalized,

$$f(t) = \boldsymbol{\Phi}_{\mathrm{b}}(\boldsymbol{I}-\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{-1}\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{P}_{\mathscr{B}\mathscr{B}}(t)\,(-\boldsymbol{Q}_{\mathscr{B}\mathscr{B}})\,\boldsymbol{G}_{\mathscr{B}\mathscr{A}}\boldsymbol{u}_{\mathscr{A}}/[E(r)-1], \tag{3.75}$$

where the denominator is one less than the mean number of openings per burst, i.e. it is the mean number of gaps per burst, from (3.7),

$$E(r)-1 = \boldsymbol{\Phi}_{\mathrm{b}}(\boldsymbol{I}-\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{-1}\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}}\boldsymbol{u}_{\mathscr{A}}. \tag{3.76}$$

The overall mean length of gaps within bursts is, therefore,

$$m = \boldsymbol{\Phi}_{\mathrm{b}}(\boldsymbol{I}-\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{-1}\boldsymbol{G}_{\mathscr{A}\mathscr{B}}(-\boldsymbol{Q}_{\mathscr{B}\mathscr{B}}^{-1})\,\boldsymbol{G}_{\mathscr{B}\mathscr{A}}\boldsymbol{u}_{\mathscr{A}}/[E(r)-1]. \tag{3.77}$$

If there is only one open state ($k_{\mathscr{A}} = 1$) the p.d.f., (3.75), reduces to

$$f(t) = \boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{P}_{\mathscr{B}\mathscr{B}}(t)\,(-\boldsymbol{Q}_{\mathscr{B}\mathscr{B}})\,\boldsymbol{G}_{\mathscr{B}\mathscr{A}}/\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}}, \tag{3.78}$$

with mean

$$m = \boldsymbol{G}_{\mathscr{A}\mathscr{B}}(-\boldsymbol{Q}_{\mathscr{B}\mathscr{B}}^{-1})\,\boldsymbol{G}_{\mathscr{B}\mathscr{A}}/\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}}. \tag{3.79}$$

If there is only one state in $\mathscr{B}$, state j say, the p.d.f., (3.75), reduces to the simple exponential distribution

$$f(t) = -q_{jj}\mathrm{e}^{q_{jj}t}, \tag{3.80}$$

with mean

$$m = 1/(-q_{jj}). \tag{3.81}$$

(*i*) *Shut periods between bursts*

The gap between bursts, defined in figure 1, starts as soon as the last opening of a burst ends, and includes at least one sojourn in $\mathscr{C}$. Obviously experimental measurements of this quantity will have the following distribution only if all bursts originate from the same individual ion channel. If we leave aside, for a moment, the question of defining how the gap starts, it is clear that, once $\mathscr{C}$ has been reached, any number ($0, \ldots, \infty$) of oscillations $\mathscr{C} \rightarrow \mathscr{B} \rightarrow \mathscr{C}$ may occur, and will be part of the gap (see figure 1), thus giving rise to a term $[\boldsymbol{I}-\boldsymbol{G}^{*}_{\mathscr{C}\mathscr{B}}(s)\,\boldsymbol{G}^{*}_{\mathscr{B}\mathscr{C}}(s)]^{-1}$ (see (2.6)); the gap may then end by transition from $\mathscr{C}$ to $\mathscr{A}$ either directly or via $\mathscr{B}$. In the latter case the last sojourn in $\mathscr{B}$ is part of the gap between bursts; so the final term in the p.d.f. will be $[\boldsymbol{G}^{*}_{\mathscr{C}\mathscr{B}}(s)\,\boldsymbol{G}^{*}_{\mathscr{B}\mathscr{A}}(s)+\boldsymbol{G}^{*}_{\mathscr{C}\mathscr{A}}(s)]\boldsymbol{u}_{\mathscr{A}}$. These terms must be preceded by an initial section that takes account of how $\mathscr{C}$ was reached, and two approaches to this are possible.

First, we can start, as in all other cases so far, from the beginning of a burst (in this case, the burst that precedes the gap of interest). The various routes through this burst will be described by terms like those in (3.16), except that in this case we are not interested in the time spent in the burst (so we put $s = 0$ in 3.16), but we are interested in any time that may be spent in $\mathscr{B}$ following the last opening of the burst, and so $G_{\mathscr{B}\mathscr{C}}$ in (3.16) is replaced by $G^*_{\mathscr{B}\mathscr{C}}(s)$. This argument gives the Laplace transform of the p.d.f. as

$$\begin{aligned} f^*(s) &= \Phi_{\mathrm{b}}[I - G_{\mathscr{A}\mathscr{B}}G_{\mathscr{B}\mathscr{A}}]^{-1}[G_{\mathscr{A}\mathscr{B}}G^*_{\mathscr{B}\mathscr{C}}(s) + G_{\mathscr{A}\mathscr{C}}][I - G^*_{\mathscr{C}\mathscr{B}}(s)\,G^*_{\mathscr{B}\mathscr{C}}(s)]^{-1} \\ &\quad \times [G^*_{\mathscr{C}\mathscr{B}}(s)\,G^*_{\mathscr{B}\mathscr{A}}(s) + G^*_{\mathscr{C}\mathscr{A}}(s)]u_{\mathscr{A}} \\ &= \Phi_{\mathrm{b}}[I - G_{\mathscr{A}\mathscr{B}}G_{\mathscr{B}\mathscr{A}}]^{-1}[G_{\mathscr{A}\mathscr{B}}G^*_{\mathscr{B}\mathscr{C}}(s) + G_{\mathscr{A}\mathscr{C}}][G^*_{\mathscr{F}\mathscr{A}}(s)]_{\mathscr{C}\mathscr{A}}u_{\mathscr{A}}. \end{aligned} \tag{3.82}$$

The latter form involves the $k_{\mathscr{C}} \times k_{\mathscr{A}}$ submatrix that consists of the last $k_{\mathscr{C}}$ rows of $G^*_{\mathscr{F}\mathscr{A}}(s)$; this form follows from (A 2.7)–(A 2.9).

The second approach is to reverse the argument used to obtain (3.2), which describes the ways in which a burst starts, to obtain a similar description of the start of a gap between bursts. In the former case, we noted that each burst was preceded by a period in $\mathscr{C}$, and that periods in $\mathscr{C}$ occurred *only* between bursts; so we started with the equilibrium fraction of the system in each $\mathscr{C}$ state, denoted $p_{\mathscr{C}}(\infty)$. Similarly we now note that a gap between bursts must be preceded by an open ($\mathscr{A}$) period and that open periods cannot occur within a gap between bursts; we therefore now take the equilibrium fraction in each open state, $p_{\mathscr{A}}(\infty)$, as the starting point, and look at the transition rate into $\mathscr{C}$, possibly via $\mathscr{B}$ (in which case the sojourn in $\mathscr{B}$ is part of the gap between bursts). This argument, when properly normalized, gives

$$f^*(s) = \Psi_{\mathrm{g}}[Q_{\mathscr{A}\mathscr{B}}G^*_{\mathscr{B}\mathscr{C}}(s) + Q_{\mathscr{A}\mathscr{C}}][I - G^*_{\mathscr{C}\mathscr{B}}(s)\,G^*_{\mathscr{B}\mathscr{C}}(s)]^{-1}[G^*_{\mathscr{C}\mathscr{B}}(s)\,G^*_{\mathscr{B}\mathscr{A}}(s) + G^*_{\mathscr{C}\mathscr{A}}(s)]u_{\mathscr{A}}, \tag{3.83}$$

where

$$\begin{aligned} \Psi_{\mathrm{g}} &= p_{\mathscr{A}}(\infty)/p_{\mathscr{A}}(\infty)\,[Q_{\mathscr{A}\mathscr{B}}G_{\mathscr{B}\mathscr{C}} + Q_{\mathscr{A}\mathscr{C}}]u_{\mathscr{C}} \\ &= p_{\mathscr{A}}(\infty)/p_{\mathscr{A}}(\infty)\,[Q_{\mathscr{A}\mathscr{F}}G_{\mathscr{F}\mathscr{A}} - Q_{\mathscr{A}\mathscr{B}}G_{\mathscr{B}\mathscr{A}}]u_{\mathscr{A}}, \end{aligned} \tag{3.84}$$

and $\mathscr{F} = \mathscr{B} \cup \mathscr{C}$ (the latter form can be derived by arguments similar to those in Appendix 1). This result can be shown to be identical with (3.82). Both of these expressions are rather lengthy, but it can be shown that inversion of these Laplace transforms gives a much simpler and more elegant form for the p.d.f. namely,

$$\begin{aligned} f(t) &= \Psi_{\mathrm{g}}[Q_{\mathscr{A}\mathscr{F}}P_{\mathscr{F}\mathscr{F}}(t)\,Q_{\mathscr{F}\mathscr{A}} - Q_{\mathscr{A}\mathscr{B}}P_{\mathscr{B}\mathscr{B}}(t)\,Q_{\mathscr{B}\mathscr{A}}]u_{\mathscr{A}} \\ &= \Psi_{\mathrm{g}}[Q_{\mathscr{A}\mathscr{F}}\exp(Q_{\mathscr{F}\mathscr{F}}t)\,(-Q_{\mathscr{F}\mathscr{F}})\,G_{\mathscr{F}\mathscr{A}} - Q_{\mathscr{A}\mathscr{B}}\exp(-Q_{\mathscr{B}\mathscr{B}}t)\,(-Q_{\mathscr{B}\mathscr{B}})\,G_{\mathscr{B}\mathscr{A}}]u_{\mathscr{A}}, \end{aligned} \tag{3.85}$$

where $\mathscr{F} = \mathscr{B} \cup \mathscr{C}$ contains all the shut states. This form is intuitively very reasonable; the numerator measures the duration of all sojourns in $\mathscr{F}$ (first term), except for those that are spent entirely within $\mathscr{B}$ (second term), and are therefore gaps within bursts rather than gaps between bursts.

The mean length of the gap between bursts is

$$m = \Psi_{\mathrm{g}}[Q_{\mathscr{A}\mathscr{F}}(-Q^{-1}_{\mathscr{F}\mathscr{F}})\,G_{\mathscr{F}\mathscr{A}} - Q_{\mathscr{A}\mathscr{B}}(-Q^{-1}_{\mathscr{B}\mathscr{B}})\,G_{\mathscr{B}\mathscr{A}}]u_{\mathscr{A}}. \tag{3.86}$$

The p.d.f. (3.85) can be expressed as the sum of $k_{\mathscr{F}} + k_{\mathscr{B}}$ exponential terms. Of these, $k_{\mathscr{F}}$ terms have rate constants that are the eigenvalues of $-Q_{\mathscr{F}\mathscr{F}}$, and coefficients, derived as in (1.29)–(1.33), from the eigenvectors of $Q_{\mathscr{F}\mathscr{F}}$. The other $k_{\mathscr{B}}$ terms have rate constants that are the eigenvalues of $-Q_{\mathscr{B}\mathscr{B}}$, and coefficients, derived as in (1.29)–(1.33), from the eigenvectors of $Q_{\mathscr{B}\mathscr{B}}$.

A special case of interest concerns mechanisms in which direct transition from $\mathscr{B}$ to $\mathscr{C}$ and back is impossible (e.g. the simple channel block mechanism, (1.9)). In this case (3.85) reduces to

$$f(t) = \boldsymbol{p}_{\mathscr{A}}(\infty)\, \boldsymbol{Q}_{\mathscr{A}\mathscr{C}} \boldsymbol{P}_{\mathscr{C}\mathscr{C}}(t)\, (-\boldsymbol{Q}_{\mathscr{C}\mathscr{C}})\, \boldsymbol{u}_{\mathscr{C}} / \boldsymbol{p}_{\mathscr{A}}(\infty)\, \boldsymbol{Q}_{\mathscr{A}\mathscr{C}} \boldsymbol{u}_{\mathscr{C}}. \tag{3.87}$$

The gap between bursts must, in this case, consist (as is clear from inspection of figure 1) of a single sojourn in $\mathscr{C}$. In this case, several of the exponential terms in (3.85) have zero coefficients, and we are left with $k_{\mathscr{C}}$ exponential terms with rate constants that are the eigenvalues of $-\boldsymbol{Q}_{\mathscr{C}\mathscr{C}}$, as is clear from (3.87) and (1.29)–(1.33). That (3.87) is the p.d.f. for the length of a single sojourn in $\mathscr{C}$ also follows directly from the results of Colquhoun & Hawkes (1981).

(i) *Another view of the initial vector* $\boldsymbol{\Phi}_{\mathrm{b}}$

It may be noted at this point that, if we set $s = 0$ in the central section of (3.82), we obtain a $(k_{\mathscr{A}} \times k_{\mathscr{A}})$ matrix, $\boldsymbol{Z}_{\mathscr{A}\mathscr{A}}$ say, of transition probabilities that describe the routes from the start of one burst to the start of the next burst. This is

$$\boldsymbol{Z}_{\mathscr{A}\mathscr{A}} = (\boldsymbol{I} - \boldsymbol{G}_{\mathscr{A}\mathscr{B}} \boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{-1} (\boldsymbol{G}_{\mathscr{A}\mathscr{B}} \boldsymbol{G}_{\mathscr{B}\mathscr{C}} + \boldsymbol{G}_{\mathscr{A}\mathscr{C}})\, (\boldsymbol{I} - \boldsymbol{G}_{\mathscr{C}\mathscr{B}} \boldsymbol{G}_{\mathscr{B}\mathscr{C}})^{-1} (\boldsymbol{G}_{\mathscr{C}\mathscr{B}} \boldsymbol{G}_{\mathscr{B}\mathscr{A}} + \boldsymbol{G}_{\mathscr{C}\mathscr{A}}). \tag{3.88}$$

Since $\boldsymbol{\Phi}_{\mathrm{b}}$, as derived in (3.2), describes the probability that a burst will start in each of the $\mathscr{A}$ states, we expect that $\boldsymbol{\Phi}_{\mathrm{b}}$ should be the same for each burst. This is so because it can be shown (by methods similar to those used in Appendix 1) that

$$\boldsymbol{\Phi}_{\mathrm{b}} \boldsymbol{Z}_{\mathscr{A}\mathscr{A}} = \boldsymbol{\Phi}_{\mathrm{b}}. \tag{3.89}$$

(*j*) *The distribution of all shut times*

It is of interest to compare the distribution of *all* shut times, both within and between bursts, with (3.85). This can be obtained directly from equations (1.24), (1.27) and (1.28) of Colquhoun & Hawkes (1981), which give the p.d.f. of the lifetime in any specified subset of states. The subset of interest now is the subset, $\mathscr{F}$, of all shut states, and its complement is $\mathscr{A}$, which contains all open states. The required p.d.f. is therefore

$$f(t) = \frac{\boldsymbol{p}_{\mathscr{A}}(\infty)\, \boldsymbol{Q}_{\mathscr{A}\mathscr{F}}}{\boldsymbol{p}_{\mathscr{A}}(\infty)\, \boldsymbol{Q}_{\mathscr{A}\mathscr{F}} \boldsymbol{u}_{\mathscr{F}}} \exp(\boldsymbol{Q}_{\mathscr{F}\mathscr{F}} t)\, (-\boldsymbol{Q}_{\mathscr{F}\mathscr{F}})\, \boldsymbol{u}_{\mathscr{F}}, \tag{3.90}$$

the sum of $k_{\mathscr{F}}$ exponential terms, with rate constants that are the eigenvalues of $-\boldsymbol{Q}_{\mathscr{F}\mathscr{F}}$, and coefficients that can be found as in (1.29)–(1.33). The mean shut lifetime is

$$m = \frac{\boldsymbol{p}_{\mathscr{A}}(\infty)\, \boldsymbol{Q}_{\mathscr{A}\mathscr{F}}}{\boldsymbol{p}_{\mathscr{A}}(\infty)\, \boldsymbol{Q}_{\mathscr{A}\mathscr{F}} \boldsymbol{u}_{\mathscr{F}}} (-\boldsymbol{Q}_{\mathscr{F}\mathscr{F}})^{-1} \boldsymbol{u}_{\mathscr{F}}. \tag{3.91}$$

(i) *Inferences about bursts from the distribution of all shut times*

The distribution of all shut times, in (3.90), is a weighted combination of the distribution of shut times between bursts, (3.85), and of the distribution of shut times within bursts, (3.75). The weights are, respectively, θ and $1-\theta$, where $\theta = 1/E(r)$ is (see (3.7)) the probability that a gap is between, rather than within, a burst. When this combination is formed it is found that the terms with rate constants that are the eigenvalues of $-\boldsymbol{Q}_{\mathscr{B}\mathscr{B}}$ in (3.75) cancel exactly with the corresponding terms in (3.85). Thus the result, the distribution of all shut times (3.90), has only terms with rate constants that are the eigenvalues of $-\boldsymbol{Q}_{\mathscr{F}\mathscr{F}}$. This is

illustrated numerically at the end of §4. It follows that the p.d.f. of all shut times, (3.90), can be written as

$$f(t) = \theta(-\boldsymbol{Q}_{\mathscr{F}\mathscr{F}} \text{ terms of p.d.f. of gaps between bursts}). \tag{3.92}$$

If the entire observed record comes from the same ion channel it is, in principle, neither necessary nor desirable to attempt to discriminate bursts of openings; the inferences should be made directly from the distributions of all shut times, and of all open times. Nevertheless the problem of inference is certainly easier if we can deal with submatrices of $\boldsymbol{Q}$ that are as small as possible, and so even when all observations are from one channel it may be thought desirable to attempt to infer the characteristics of shut periods within, and between bursts of openings, by inspection of the p.d.f. of all shut periods. However, such inferences cannot be exact. The rate constants for the p.d.f. of gaps within bursts are the eigenvalues of $-\boldsymbol{Q}_{\mathscr{B}\mathscr{B}}$, but these will not, in general, be the same as the rate constants for the faster components of the distribution of all shut times, because the latter has rate constants that are the eigenvalues of $-\boldsymbol{Q}_{\mathscr{F}\mathscr{F}}$ (i.e., since $\mathscr{F} = \mathscr{B} \cup \mathscr{C}$, they involve $\mathscr{C}$ states as well as $\mathscr{B}$ states). Similarly the area under the slowest component(s) of the distribution of all shut times cannot, in general, be equated with the fraction (denoted θ above) of gaps that are between rather than within bursts. Nevertheless, in particular cases the inferences of the length and number of intraburst gaps from the distribution of all shut periods may be a good approximation, as in the example discussed in §4 (see (4.25)–(4.28)). For example, if the distribution of the gap between bursts, $f_b(t)$ say, is well approximated by a single exponential term, with a rate constant that is an eigenvalue (λ_1 say) of $-\boldsymbol{Q}_{\mathscr{F}\mathscr{F}}$, then this distribution will be approximately of the form

$$f_b(t) \approx \lambda_1 e^{-\lambda_1 t}. \tag{3.93}$$

Thus, from (3.92), the term with this rate constant in the distribution of all shut times will have a coefficient, w_1, say, of

$$w_1 \approx \theta\lambda_1. \tag{3.94}$$

Therefore the area under the slow component of the distribution of all shut times, w_1/λ_1, will be *approximately* θ the fraction of all shut times that are between bursts rather than within bursts.

4. A NUMERICAL EXAMPLE

(a) *The mechanism and its parameter values*

The general results for analysis of bursts, which were derived in the preceding section, will now be illustrated by the mechanism that was introduced in (1.11). In this mechanism, two agonist molecules (A) can bind to the shut (T) conformation, and either singly or doubly occupied receptor ion channels may open (R). The (microscopic) rate constants for the mechanism are defined as follows:

state number:

$$\begin{array}{lccc} 5 & \mathrm{T} & & \\ & {\scriptstyle k_{+1}}\big\uparrow\big\downarrow{\scriptstyle k_{-1}} & & \\ 4 & \mathrm{AT} & \underset{\alpha_1}{\overset{\beta_1}{\rightleftharpoons}} & \mathrm{AR} \quad 1 \\ & {\scriptstyle k_{+2}}\big\uparrow\big\downarrow{\scriptstyle k_{-2}} \qquad {\scriptstyle k^*_{+2}}\big\uparrow\big\downarrow{\scriptstyle k^*_{-2}} & & \\ 3 & \mathrm{A_2T} & \underset{\alpha_2}{\overset{\beta_2}{\rightleftharpoons}} & \mathrm{A_2R} \quad 2. \end{array} \tag{4.1}$$

The open states, 1 and 2, constitute the subset $\mathscr{A}$; so $k_{\mathscr{A}} = 2$. When the agonist concentration (denoted x_A) is low, sojourns in state 5 (T) will be long; so this will be defined as the sole member of the subset $\mathscr{C}$ ($k_{\mathscr{C}} = 1$). Open periods may be expected, in general, to be interrupted by short sojourns in the occupied but shut states, 3 and 4 (A_2T and AT), which therefore constitute the subset $\mathscr{B}$ ($k_{\mathscr{B}} = 2$).

The matrix of transition rates, partitioned as in (1.6), is

$$Q = \begin{array}{c} \\ 1 \\ 2 \\ 3 \\ 4 \\ 5 \end{array} \left[\begin{array}{ccc|cc|c} & 1 & 2 & 3 & 4 & 5 \\ \hline 1 & -(\alpha_1 + k^*_{+2}x_A) & k^*_{+2}x_A & 0 & \alpha_1 & 0 \\ 2 & 2k^*_{-2} & -(\alpha_2 + 2k^*_{-2}) & \alpha_2 & 0 & 0 \\ \hline 3 & 0 & \beta_2 & -(\beta_2 + 2k_{-2}) & 2k_{-2} & 0 \\ 4 & \beta_1 & 0 & k_{+2}x_A & -(\beta_1 + k_{+2}x_A + k_{-1}) & k_{-1} \\ \hline 5 & 0 & 0 & 0 & 2k_{+1}x_A & -2k_{+1}x_A \end{array}\right]. \tag{4.2}$$

The numerical values for the rate constants that will be used for this example have been chosen so that the predictions are similar, in most respects, to the observations described by Colquhoun & Sakmann (1981), who used suberyldicholine as agonist. However it should be emphasized that further experiments are necessary before the qualitative mechanism in (4.1) (and, *a fortiori*, the values for the rate constants) can be regarded as secure. The values are as follows.

(1) First binding: $k_{+1} = 5 \times 10^7$ M^{-1} s^{-1}, $k_{-1} = 2000$ s^{-1}, and so the equilibrium constant is $K_1 = 40$ μM. The observations contain little information about k_{+1} because of the low agonist concentration, and because the gaps between bursts are not interpretable as a result of there being several ion channels contributing to the observed record. However a rough guess can be made from the concentration of agonist required for a given current (number of open channels) at equilibrium.

(2) Second binding to the shut conformation: $k_{+2} = 5 \times 10^8$ M^{-1} s^{-1}, $k_{-2} = 2000$ s^{-1}, and so the equilibrium constant is $K_2 = 4$ μM. Again the value for the association rate constant is somewhat arbitrary, but a rather fast value is needed to make ϕ_2 large enough relative to ϕ_1 (see (4.6) and (4.7) below).

(3) Opening of the singly occupied state: $\beta_1 = 15$ s^{-1}, $\alpha_1 = 3000$ s^{-1}.

(4) Opening of the doubly occupied state: $\beta_2 = 15000$ s^{-1}, $\alpha_2 = 500$ s^{-1}.

(5) Binding to the open state. It follows from microscopic reversibility that the values already given imply an equilibrium constant of 0.66 nM. If we arbitrarily assume that the association rate constant, k^*_{+2}, is the same as k_{+2}, i.e. 5×10^8 M^{-1} s^{-1}, then the equilibrium constant implies that $k^*_{-2} = 0.33$ s^{-1}. Thus dissociation from the doubly occupied open form would be very slow (time constant 3 s).

(6) Agonist concentration, $x_A = 100$ nM; so $k_{+1}x_A = 5$ s^{-1} and $k_{+2}x_A = 50$ s^{-1}. At this very low agonist concentration, bursts of openings are well separated, and therefore clearly defined. Furthermore, at low agonist concentration the parameters (such as time constants and relative areas) of the observed distributions (of gap length etc.) often take on, to a good approximation, a simple physical significance which they will not, in general, have at higher concentrations.

The equilibrium constants just given are not unlike those suggested by Dionne *et al.* (1978)

as a possible interpretation of their equilibrium experiments with carbachol, except that the binding affinity for carbachol was lower, and it appeared that carbachol could open, at most, around 50% of the channels. According to the numbers just given, suberyldicholine appears to be a rather efficacious agonist in that it could, in principle, open a fraction $\beta_2/(\alpha_2+\beta_2)$, i.e. 96.8%, of channels at high enough concentration. The equilibrium constant for opening when doubly liganded is $\beta_2/\alpha_2 = 15000/500 = 30$, which is 6000 times greater than when singly liganded ($\beta_1/\alpha_1 = 0.005$). It is intriguing to speculate that, if the same ratio held for unliganded channels, as in the Monod–Wyman–Changeux model, the probability of a channel opening in the absence of any agonist would be 0.005/6000 i.e. about one in a million, or about ten channels per endplate.

(i) *Equilibrium state occupancies*

The fractions of the system in each state at equilibrium are: $p_1(\infty) = 2.48 \times 10^{-5}$, $p_2(\infty) = 1.86 \times 10^{-3}$ (so total fraction open is 1.89×10^{-3}, of which 98.7% are doubly occupied), $p_3(\infty) = 6.21 \times 10^{-5}$, $p_4(\infty) = 4.97 \times 10^{-3}$, and $p_5(\infty) = 0.9931$.

(ii) *The Q and Π matrices*

From the numerical values just given, and (4.2), we have

$$Q = \left[\begin{array}{cc|cc|c} -3050 & 50 & 0 & 3000 & 0 \\ 0.667 & -500.667 & 500 & 0 & 0 \\ \hline 0 & 15000 & -19000 & 4000 & 0 \\ 15 & 0 & 50 & -2065 & 2000 \\ \hline 0 & 0 & 0 & 10 & -10 \end{array}\right] \tag{4.3}$$

The diagonal elements give the mean lifetimes of single sojourns in each individual state as:

$$\left.\begin{array}{ll} \text{state 1 (AR),} & 0.328 \text{ ms;} \\ 2 \text{ (A}_2\text{R),} & 1.997 \text{ ms;} \\ 3 \text{ (A}_2\text{T),} & 52.6 \ \mu\text{s;} \\ 4 \text{ (AT),} & 0.484 \text{ ms;} \\ 5 \text{ (T),} & 100 \text{ ms.} \end{array}\right\} \tag{4.4}$$

From (4.3) and (1.5), the transition probabilities, π_{ij}, are given by

$$\Pi = \begin{bmatrix} — & 0.0164 & 0 & 0.9836 & 0 \\ 0.0013 & — & 0.9987 & 0 & 0 \\ 0 & 0.7895 & — & 0.2105 & 0 \\ 0.0073 & 0 & 0.0242 & — & 0.9685 \\ 0 & 0 & 0 & 1 & — \end{bmatrix} \tag{4.5}$$

Thus, for example, a channel in state 4 (AT), has a 0.7% chance that its next transition is to open (to state 1, AR), a 2.4% chance of binding another agonist molecule (transition to state 3, A_2T), and a 96.9% chance of losing its agonist molecule (transition to state 5, T).

(iii) *The predicted noise spectrum*

The methods of Colquhoun & Hawkes (1977) allow us to predict that the spectral density function for fluctuations in the number of open channels should consist of the sum of $k-1 = 4$ Lorentzian components with time constants that are the reciprocals of the eigenvalues of $-\boldsymbol{Q}$. (The fifth eigenvalue is zero because $\boldsymbol{Q}$ is always singular.) These are 9.82 ms, 0.494 ms, 0.323 ms and 51.5 μs. The relative amplitudes of these components are, respectively, 100, 0.0009, 0.046 and 0.011; thus only the first component with a time constant of 9.82 ms would be observable in practice. It will be shown below that this is close to the time constant of the slow component of the distribution of the burst length (as in the example given in Colquhoun & Hawkes 1977).

(b) The start of a burst

The probability that a burst starts in each open state is given by (3.2). In this mechanism there is only one state in $\mathscr{A} \cup \mathscr{B}$ that is accessible from $\mathscr{C}$ (special case 3, §3*a*); so $\boldsymbol{\Phi}_B$ is the second row of $\boldsymbol{G}_{\mathscr{B}\mathscr{A}}$ scaled to sum to unity. This gives the probability of a burst starting in the doubly liganded state, A_2R (state 2), as

$$\phi_2 = \beta_2 k_{+2} x_A / [\beta_2 k_{+2} x_A + \beta_1(\beta_2 + 2k_{-2})] = 0.725 \tag{4.6}$$

Likewise the probability that a burst starts in the singly liganded state, AR (state 1), is

$$\phi_1 = 1 - \phi_2 = 0.275. \tag{4.7}$$

(c) The number of openings per burst

From (1.25) we find

$$\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}} = \begin{bmatrix} 0.00721 & 0.0319 \\ 0.00154 & 0.7925 \end{bmatrix}. \tag{4.8}$$

Clearly most $\mathscr{A} \to \mathscr{B} \to \mathscr{A}$ transitions start and end in state 2 (A_2R). The distribution of the number of openings per burst is the sum of two geometric distributions, as in (3.9). The probability of there being r openings is

$$P(r) = 0.153\rho_1^{r-1} + 0.261\rho_2^{r-1}, \tag{4.9}$$

with mean

$$E(r) = 3.82. \tag{4.10}$$

The coefficients are given by (3.10), and ρ_1 and ρ_2, the eigenvalues of (4.8), are 0.7926 and 0.00714 respectively. The second component dies out rapidly in this case. From (4.9) we find that 41.4% of bursts should have only one opening, 12.3% should have two openings, and 1.9% should have ten openings. The mean is 3.82 openings per burst.

In general, if a distribution that actually consists of the sum of two geometric distributions is fitted by a single geometric distribution with the same mean, it will appear that the observations contain too many bursts with small and too many with large numbers of openings, but too few with intermediate numbers of openings. Deviations of this sort from a single geometric distribution were frequently observed by D. Colquhoun & B. Sakmann (unpublished results).

The reasons for the shape of this distribution are made clearer by consideration of the distributions conditional on starting state, given by (3.13). These have, of course, the same values of ρ_1 and ρ_2 as above. But, for bursts that start in state 1 (AR), the coefficients are 0.00844 and 0.952 respectively, and so the second component predominates; the mean is 1.16 openings

per burst, and most bursts that start in AR consist of a single sojourn in AR (as is shown by their mean length; see below). For bursts that start in state 2 (A_2R) the coefficients are 0.208 and $-0.001\,87$ respectively, and so the first component predominates; the mean is 4.82 openings per burst and, as is shown below, most bursts will, in this case, consist of oscillations between A_2T and A_2R. It is clear from (4.5) that oscillations between AR and A_2R will be rather rare in this example.

(d) The burst length

It follows from (3.17) that the p.d.f. of the burst length is the sum of four exponential terms, with rate constants that are the eigenvalues of $-Q_{\mathscr{E}\mathscr{E}}$, i.e. the top left 4×4 section of (4.3). These, with their reciprocals, are:

$$\left.\begin{aligned} \lambda_1 &= 101.6\ \text{s}^{-1}, & \tau_1 &= 9.84\ \text{ms};\\ \lambda_2 &= 2013\ \text{s}^{-1}, & \tau_2 &= 0.497\ \text{ms};\\ \lambda_3 &= 3093\ \text{s}^{-1}, & \tau_3 &= 0.323\ \text{ms};\\ \lambda_4 &= 19408\ \text{s}^{-1}, & \tau_4 &= 51.5\ \mu\text{s}. \end{aligned}\right\} \tag{4.11}$$

Because the agonist concentration is so low, these are almost identical to the values expected (see above) for the noise spectrum. The p.d.f. of the burst length, the areas under each of the four components and the mean are:

$$\left.\begin{aligned} f(t) &= 74.7\ \text{e}^{-\lambda_1 t} + 28.7\ \text{e}^{-\lambda_2 t} + 774\ \text{e}^{-\lambda_3 t} + 1.50\ \text{e}^{-\lambda_4 t};\\ \text{area} &= 0.736 + 0.014 + 0.250 + 0.000 = 1.000;\\ \text{mean} &= 7.33\ \text{ms}. \end{aligned}\right\} \tag{4.12}$$

Only the first and third components are big enough to be easily observable; about three-quarters of the area is accounted for by the slow component ($\tau_1 = 9.84$ ms), and most of the rest by a faster component ($\tau_3 = 0.323$ ms). The physical interpretation of this result is, in this example, clear. The mean burst length is close to the mean number of openings per burst (3.82) times the overall mean length of an opening (1.88 ms; see table 1) (plus 2.82 intraburst gaps of 57.6 μs, though these contribute only a small amount to the burst length). The slow component ($\tau_1 = 9.84$ ms) corresponds approximately with the mean number of openings per burst given that the burst starts in state 2 (A_2R), namely 4.82 (see above), multiplied by the corresponding open time, 2.00 ms (which is close to the mean lifetime of the doubly occupied open state, A_2R; see (4.4) and table 1). Bursts that start in AR, on the other hand, will rarely have more than one opening (mean 1.16), with mean life that of AR, 0.328 ms (see (4.4)); this is close to the time constant, 0.323 ms, of the fast component of the burst distribution. In view of the simple physical interpretation that can be placed on the burst length distribution in this particular example, it is not surprising that the proportion of the area under the slow component of the distribution is close to the proportion of bursts that start in A_2R (i.e. $\phi_2 = 0.725$, from (4.6)).

(e) The total open time per burst

The eigenvalues of $-V_{\mathscr{A}\mathscr{A}}$ (defined in (3.24)) are $\lambda_1 = 103.9\ \text{s}^{-1}$ ($\tau_1 = 9.63$ ms), and $\lambda_2 = 3028\ \text{s}^{-1}$ ($\tau_2 = 0.330$ ms). The p.d.f. of the total open time per burst, from (3.23), is

$$\left.\begin{aligned} f(t) &= 76.3\ \text{e}^{-\lambda_1 t} + 802\ \text{e}^{-\lambda_2 t};\\ \text{area} &= 0.735 + 0.265 = 1.000;\\ \text{mean} &= 7.17\ \text{ms}. \end{aligned}\right\} \tag{4.13}$$

This distribution is similar to that of the total burst length (4.12), as might be expected from the shortness of the gaps within bursts in this example (see table 2).

(f) The total shut time per burst

The eigenvalues of $-\boldsymbol{W}_{\mathscr{B}\mathscr{B}}$ (defined in (3.36)) are $\lambda_1 = 1953\ s^{-1}$ ($\tau_1 = 0.512$ ms) and $\lambda_2 = 4117\ s^{-1}$ ($\tau_2 = 0.243$ ms). The p.d.f. of the total duration of all gaps within a burst, for bursts that contain at least one gap, from (3.40), is

$$\left.\begin{aligned} f'(t) &= 246\, e^{-\lambda_1 t} + 3598\, e^{-\lambda_2 t}; \\ \text{area} &= 0.126 + 0.874 = 1.00; \\ \text{mean} &= 277\ \mu s. \end{aligned}\right\} \tag{4.14}$$

Multiplication of the mean by the probability that a burst contains at least one gap, i.e. $1 - 0.414$ (see above), gives the mean shut time for all bursts, from (3.42), as $m = 162$ μs. This is the mean gap length, 57.6 μs (see table 2), multiplied by the mean number of gaps per burst, 2.82 (see (4.10)).

(g) The length of individual openings

In the observations of Colquhoun & Sakmann (1981) a substantial proportion of gaps within bursts were too short to resolve; so the length of individual openings could not be measured satisfactorily. Nevertheless it is of interest to see what the predictions are in this example.

All the distributions of open times to be discussed are the sum of two ($k_{\mathscr{A}}$) exponential components, with amplitudes denoted w_1, w_2, i.e.

$$f(t) = w_1 e^{-\lambda_1 t} + w_2 e^{-\lambda_2 t}. \tag{4.15}$$

This can, alternatively, be written in the form

$$f(t) = \theta_1 \lambda_1 e^{-\lambda_1 t} + (1 - \theta_1)\lambda_2 e^{-\lambda_2 t}, \tag{4.16}$$

in which $\theta_1 = w_1/\lambda_1$ denotes the fraction of the area under the p.d.f. that is contributed by the slower (λ_1) component. The amplitudes and areas differ from one distribution to another, but all of the distributions have the same rate constants, the eigenvalues of $-\boldsymbol{Q}_{\mathscr{A}\mathscr{A}}$:

$$\left.\begin{aligned} \lambda_1 &= 500.6\ s^{-1} \quad (\tau_1 = 2.00\ \text{ms}); \\ \lambda_2 &= 3050\ s^{-1} \quad (\tau_2 = 0.328\ \text{ms}). \end{aligned}\right\} \tag{4.17}$$

In this case, because of the low agonist concentration and slow dissociation of agonist from A_2R, these two time constants are very close to the mean lifetimes of states A_2R and AR, respectively.

Table 1*a* gives the distributions and means of the p.d.f. of the kth opening in a burst with r openings, from (3.52) and (3.53). The distributions show the time symmetry discussed following (3.53).

The distribution (3.57) of the length of the kth opening in a burst, regardless of the number of openings, is given in table 1*b*. Note that, in both of these cases, bursts with few openings tend to have short openings; this is because, as already found, there is little interchange between AR and A_2R and so bursts with few openings tend to consist of a single sojourn in AR, whereas bursts with many openings will usually be those that started in A_2R.

The rarity of interchange between AR and A_2R during a single opening is reflected in distributions that are conditional on the initial state. For example, table 1*c*, *d* shows the distributions of the duration of single openings, regardless of position within the burst, given that

the opening occurs in a *burst* that started in AR (table 1*c*), or in state A_2R (table 1*d*). The former are, on average, shorter. These distributions are found by omission of $\mathbf{\Phi}_b$ from (3.60).

Another, related, approach is to calculate the p.d.f. of the length of an opening (regardless of position in burst) conditional on the *opening* starting in a specified state. This can be done by omission of $\mathbf{\Phi}_0$ from (3.64). For openings that start in AR we find that the term with time constant $\tau_2 = 0.328$ ms accounts for 98 % of the area under the p.d.f., and the mean length of such openings is 0.361 ms, which is only slightly greater than the mean length, 0.328 ms, of a single sojourn in AR. And for openings that start in A_2R the p.d.f. is very nearly a single exponential with mean close to $\tau_2 = 2.00$ ms, which is almost the same as the mean length of a single sojourn in A_2R.

TABLE 1. THE DISTRIBUTION OF OPEN LIFETIME

(All the distributions have the same two rate constants, specified in (4.17). The contributions of each of these two components is specified here both in terms of their amplitudes, w_1 and w_2 (see (4.15)), and in terms of the relative area of the slow components, θ_1 (see (4.16)).)

			amplitudes/s^{-1}		
k	r	area, θ_1	w_1	w_2	mean/ms
		(*a*) The kth opening in a burst with r openings			
1	1	0.364	182	1941	0.935
1	2	0.977	489	70.0	1.96
2	2	0.977	489	70.0	1.96
1	3	0.992	497	24.4	1.98
2	3	1.00	501	0.962	2.00
3	3	0.992	497	24.4	1.98
		(*b*) The kth opening in a burst (regardless of r)			
1	—	0.730	366	823	1.55
2	—	0.995	498	15.1	1.99
3	—	0.998	500	5.18	1.99
		(*c*) Any opening in a burst that started in AR			
		0.150	75.2	2592	0.579
		(*d*) Any opening in a burst that started in A_2R			
		0.999	500	3.86	2.00
		(*e*) Overall distribution			
		0.928	464	221	1.88

The overall distribution of open time, from (3.60) or (3.64), is given in table 1*e*; the mean, 1.88 ms, is only slightly less than the mean lifetime of A_2R, because the area under the slow (2 ms) component of the p.d.f. is 92.8 % of the total area. This is close to the probability that any opening starts in A_2R, which is given in $\mathbf{\Phi}_0$. From (3.63),

$$\mathbf{\Phi}_0 = [\beta_1 2k_{-2}/d \quad \beta_2 k_{+2} x_A/d], \tag{4.18}$$

where

$$d = \beta_1 2k_{-2} + \beta_2 k_{+2} x_A. \tag{4.19}$$

In this case therefore

$$\mathbf{\Phi}_0 = [0.074 \quad 0.926]. \tag{4.20}$$

The probability that *any* individual opening starts in A_2R (0.926, from (4.20)) is greater than the probability that the first opening in burst starts in A_2R (0.725, from (4.6)). This is because a burst must be preceded by a sojourn in state 5 (T), and so it is always necessary to go through

state AT (with the possibility of opening to AR) before a burst starts. On the other hand the second and subsequent openings in a burst may be preceded by gaps spent only in A_2T.

(*h*) *The length of gaps within a burst*

All of the distributions, which are summarized in table 2, are the sums of two ($k_{\mathscr{B}}$) exponential components, of the form already specified in (4.15) and (4.16).

All the distributions have the same rate constants, λ_1, λ_2, which are the eigenvalues of $-Q_{\mathscr{B}\mathscr{B}}$, namely

$$\left.\begin{aligned}\lambda_1 &= 2053\ \text{s}^{-1} \quad (\tau_1 = 0.487\ \text{ms}),\\ \lambda_2 &= 19012\ \text{s}^{-1} \quad (\tau_2 = 52.6\ \mu\text{s}).\end{aligned}\right\} \tag{4.21}$$

In this example, because of the low agonist concentration, these time constants are close to the mean lifetimes of the two occupied but shut states, AT and A_2T respectively (see (4.4)).

TABLE 2. THE DISTRIBUTIONS OF THE LENGTH OF GAPS WITHIN BURSTS

(All distributions have the same two rate constants, given in (4.21). The contributions of each of these two components are specified here both in terms of their amplitudes, w_1 and w_2 (see (4.15)), and in terms of the relative area of the slow component, θ_1 (see (4.16)).)

			amplitudes/s^{-1}		
k	r	area, θ_1	w_1	w_2	mean/μs
		(*a*) The kth gap in a burst with r openings			
1	2	0.041	84.8	18227	70.5
1	3	0.017	34.6	18692	59.9
2	3	0.017	34.6	18692	59.9
1	4	0.017	34.1	18696	59.8
2	4	0.007	13.9	18883	55.5
3	4	0.017	34.1	18696	59.8
		(*b*) The kth gap in a burst (regardless of r)			
1	—	0.022	44.8	18597	62.1
2	—	0.009	18.1	18845	56.4
3	—	0.009	17.8	18847	56.4
		(*c*) Any gap within a burst that started in AR			
		0.183	376	15528	132.0
		(*d*) Any gap within a burst that started in A_2R			
		0.009	17.8	18847	56.4
		(*e*) Overall distribution			
		0.011	23.5	18794	57.6

The p.d.f. of the length of the kth gap in a burst with r openings from (3.69) is shown in table 2*a*. They show the time symmetry discussed earlier. The kth gap in a burst (table 2*b*) also shows a slight tendency for gaps to be longer in bursts with few openings, because bursts with few openings will often be those that started in AR, and so, if they have a gap, it will usually be in AT. This is also shown by the distributions of the length of intraburst gaps (regardless of position in the burst), given that the gap is part of a burst that started in AR (table 2*c*) or a burst that started in A_2R (table 2*d*). The latter have gaps with mean length close to the mean life of a single sojourn in A_2T, but the former have longer gaps on average. These distributions are found by omission of Φ_b from (3.75).

The overall distribution of the length of gaps within bursts (table 2*e*) is dominated by the

fast component with time constant close to the mean lifetime of A_2T, which accounts for 98.9 % of the area under the p.d.f. However, there is a small component (1.1 % of area) with a longer time constant, 0.487 ms, which is close to the mean lifetime of AT. A small component, qualitatively resembling this, was observed by Colquhoun & Sakmann (1981), but it is not possible, at present, to be sure that it should be interpreted in the way just described.

(*i*) *The gaps between bursts*

The gaps between bursts could not be properly measured by Colquhoun & Sakmann (1981) because more than one ion channel contributed to their records. The gap of 300–1000 ms that was common in their experiments will therefore, in general, be shorter than the true gap between bursts (which, in this example, is predicted to be 3790 ms).

The distribution, from (3.85), consist of five $(k_{\mathscr{B}}+k_{\mathscr{F}})$ exponential components. The first two (λ_1, λ_2) have rate constants that are eigenvalues of $-\boldsymbol{Q}_{\mathscr{B}\mathscr{B}}$, which have already been given in (4.21). They are

$$\lambda_1 = 2053\ \text{s}^{-1} \quad (\tau_1 = 0.487\ \text{ms}),$$

$$\lambda_2 = 19012\ \text{s}^{-1} \quad (\tau_2 = 52.6\ \mu\text{s}).$$

The other three components have rate constants that are the eigenvalues of $-\boldsymbol{Q}_{\mathscr{F}\mathscr{F}}$, where $\mathscr{F} = \mathscr{B} \cup \mathscr{C}$, which are

$$\left.\begin{aligned} \lambda_3 &= 0.2639\ \text{s}^{-1} \quad (\tau_3 = 3789\ \text{ms}),\\ \lambda_4 &= 2063\ \text{s}^{-1} \quad (\tau_4 = 0.485\ \text{ms}),\\ \lambda_5 &= 19012\ \text{s}^{-1} \quad (\tau_5 = 52.6\ \mu\text{s}). \end{aligned}\right\} \tag{4.22}$$

Because of the low agonist concentration λ_1 and λ_4 are nearly equal, as are λ_2 and λ_5. These components nearly cancel in the overall p.d.f., which is

$$\left.\begin{aligned} f_{\text{b}}(t) &= -66.2\ \text{e}^{-\lambda_1 t} - 52975\ \text{e}^{-\lambda_2 t} + 0.264\ \text{e}^{-\lambda_3 t} + 65.9\ \text{e}^{-\lambda_4 t} + 52975\ \text{e}^{-\lambda_5 t};\\ \text{mean} &= 3790\ \text{ms}. \end{aligned}\right\} \tag{4.23}$$

Therefore the distribution is close to a single exponential distribution, consisting only of the λ_3 term.

The mean length of a gap between bursts is much longer than the mean length of a sojourn in $\mathscr{C}$ (state T), which, from (4.4), is only 100 ms. This is because there may be many occupancies, as long as they do not lead to opening, between bursts (see figure 1). Most of these will be single occupancies; from (4.5) it is seen that state AT has a 96.9 % chance of dissociating rather than opening or becoming doubly occupied. But, if double occupancy is attained, then, from (4.5), A_2T has a 78.9 % chance of opening.

(*j*) *The distribution of all shut times*

The distribution of all shut times, like that of gaps between bursts, could not be properly measured by Colquhoun & Sakmann (1981), because of the presence of more than one ion channel.

The distribution, from (3.90), consists of $k_{\mathscr{F}} = 3$ exponential components with rate constants that are the eigenvalues, λ_3, λ_4 and λ_5, of $-\boldsymbol{Q}_{\mathscr{F}\mathscr{F}}$, which have been given in (4.22). It is

$$\left.\begin{aligned} f_{\text{s}}(t) &= 0.06913\ \text{e}^{-\lambda_3 t} + 17.26\ \text{e}^{-\lambda_4 t} + 13873\ \text{e}^{-\lambda_5 t};\\ \text{area} &= 0.2619 + 0.0084 + 0.7297 = 1.000;\\ \text{mean} &= 992.7\ \text{ms}. \end{aligned}\right\} \tag{4.24}$$

This distribution can, as discussed at the end of §3, be expressed as a weighted combination of the distribution, $f_b(t)$, of gaps between bursts, (4.23), and of the overall distribution, $f_w(t)$, of gaps within bursts which, from table 2*e*, is

$$\left.\begin{aligned} f_w(t) &= 23.48\ e^{-\lambda_1 t} + 18794\ e^{-\lambda_2 t}; \\ \text{area} &= 0.011 + 0.989 = 1.000; \\ \text{mean} &= 57.6\ \mu s. \end{aligned}\right\} \tag{4.25}$$

In this expression, λ_1 and λ_2 are the eigenvalues of $-\boldsymbol{Q}_{\mathscr{B}\mathscr{B}}$ given in (4.21). Thus

$$f_s(t) = \theta f_b(t) + (1-\theta) f_w(t), \tag{4.26}$$

where, from (4.10), the probability that a gap is between, rather than within, bursts is

$$\theta = 1/E(r) = 1/3.82 = 0.2619. \tag{4.27}$$

Evaluation of (4.26) gives

$$\begin{aligned} f_s(t) = &-17.33\ e^{-\lambda_1 t} - 13873\ e^{-\lambda_2 t} \\ &+ 0.06913\ e^{-\lambda_3 t} + 17.26\ e^{-\lambda_4 t} + 13873\ e^{-\lambda_5 t} \\ &+ 17.33\ e^{-\lambda_1 t} + 13873\ e^{-\lambda_2 t}. \end{aligned} \tag{4.28}$$

As expected, the terms in λ_1, λ_2 cancel, leaving only those derived from $\boldsymbol{Q}_{\mathscr{F}\mathscr{F}}$ that are identical with (4.24).

(i) *Inferences about bursts from the distribution of all shut times*

One would infer from the distributions of all shut times, (4.24), that the distributions of the lengths of gaps within bursts had time constants $\tau_4 = 1/\lambda_4 = 0.485$ ms and $\tau_5 = 1/\lambda_5 = 52.6$ μs. In this example these values are very close to the correct values, which are, from (4.25) and (4.21), $\tau_1 = 1/\lambda_1 = 0.487$ ms and $\tau_2 = 1/\lambda_2 = 52.6$ μs. Similarly one would infer from (4.24) that the mean length of the gap between bursts was $\tau_3 = 1/\lambda_3 = 3789$ ms, which is very close to the correct value, from (4.23), of 3790 ms. The fraction of all gaps that are between bursts would be inferred, from (4.24), to be the area, 0.2619, under the slowest (λ_3) component; in this example the result is again very close to the correct value given in (4.27). This is what would be expected, from (3.93) and (3.94), as a result of the fact that the distribution of the gap between bursts (4.23) is, in this example, a close approximation to a single exponential component, the coefficient for the λ_3 term in (4.23) being 0.264 s^{-1} which is close to λ_3.

The very low agonist concentration in this particular example has ensured that the burst characteristics inferred from the distributions of all shut times are very close approximations to the true values. In fact, in this particular case the approximations remain quite adequate even with an agonist concentration sufficient to keep the channels open for 50% of the time. However, we cannot give general conditions that will ensure the validity of the approximation; numerical calculations of the sort given above may be necessary in each particular case.

5. The analysis of clusters of bursts

(*a*) *Basic definitions*

A procedure similar to that used for analysis of simple bursts will be followed; we now divide the k states in which the system can exist into four subsets, rather than the three subsets used previously. These are defined as follows.

(1) Subsets $\mathscr{A}$ (open states), $\mathscr{B}$ (short-lived shut states) and $\mathscr{C}$ (long-lived shut states) are defined exactly as before (§1*a*).

(2) Subset $\mathscr{D}$ comprises very long-lived shut states, $k_{\mathscr{D}}$ in number, such that an entry into $\mathscr{D}$ results in a shut period so long that it is deemed to be part of a gap between clusters.

(3) As before, we define a number of subsets that result from pooling two or more of the above subsets into one, thus:

$$\left.\begin{aligned}
\mathscr{E} &= \mathscr{A} \cup \mathscr{B} \text{ ('burst states'),}\\
\mathscr{F} &= \mathscr{B} \cup \mathscr{C} \text{ ('gap between burst, within cluster, states'),}\\
\mathscr{G} &= \mathscr{A} \cup \mathscr{B} \cup \mathscr{C} \text{ ('cluster states'),}\\
\mathscr{H} &= \mathscr{C} \cup \mathscr{D} \text{ ('gap between cluster states'), and}\\
\mathscr{T} &= \mathscr{B} \cup \mathscr{C} \cup \mathscr{D} \text{ (all shut states).}
\end{aligned}\right\} \tag{5.1}$$

In practice the discrimination between gaps that are within bursts, those that are between bursts within a cluster, and those that are between clusters will be even more prone to error than the discrimination of simple bursts (discussed at the end of §1), but this will not be discussed further here.

The grouping of bursts into clusters has been demonstrated by Sakmann *et al.* (1980). In their experiments the agonist concentration was high; so the lifetime of vacant receptors (T in (1.7) or (1.11)) would be short. Therefore sojourns in T, as well as those in the occupied but shut states (AT or A_2T), would be classified as gaps within a burst; thus all of these states would be members of the subset $\mathscr{B}$. The occurrence of bursts was attributed to the existence of a desensitized state or states (subset $\mathscr{C}$); after fluctuating rapidly between vacant, occupied but shut, and open states for a while, the burst would be ended by transition into the relatively long-lived desensitized state. The occurrence of clusters was attributed to the existence of a second, very long-lived desensitized state or states (subset $\mathscr{D}$), entry into which implied the end of a cluster of bursts.

The matrix of transition rates can now be partitioned thus:

$$Q = \begin{bmatrix} Q_{\mathscr{A}\mathscr{A}} & Q_{\mathscr{A}\mathscr{B}} & Q_{\mathscr{A}\mathscr{C}} & Q_{\mathscr{A}\mathscr{D}} \\ Q_{\mathscr{B}\mathscr{A}} & Q_{\mathscr{B}\mathscr{B}} & Q_{\mathscr{B}\mathscr{C}} & Q_{\mathscr{B}\mathscr{D}} \\ Q_{\mathscr{C}\mathscr{A}} & Q_{\mathscr{C}\mathscr{B}} & Q_{\mathscr{C}\mathscr{C}} & Q_{\mathscr{C}\mathscr{D}} \\ Q_{\mathscr{D}\mathscr{A}} & Q_{\mathscr{D}\mathscr{B}} & Q_{\mathscr{D}\mathscr{C}} & Q_{\mathscr{D}\mathscr{D}} \end{bmatrix}. \tag{5.2}$$

An example of the possible behaviour of the system is shown in figure 2, which is constructed like figure 1 except that it includes the new subset, $\mathscr{D}$, entry into which implies the end of a cluster. The terms used in this section are defined in figure 2.

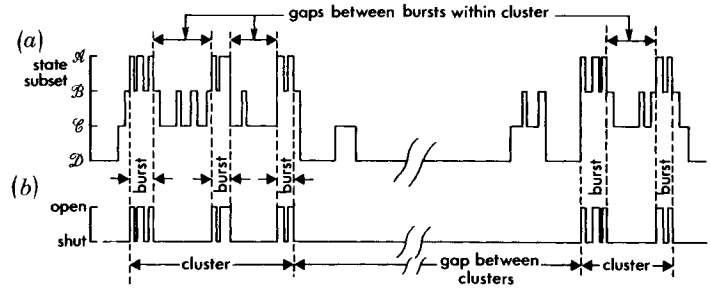

FIGURE 2. Diagrammatic representation of possible behaviour of a single ion channel, which has any mechanism that results in bursts of openings occurring in well defined clusters. The upper part shows transitions of the system between the four subsets of states defined at the beginning of §5. These are the same as for figure 1, with the addition of a subset, $\mathscr{D}$, of shut states with lives so long that entry into $\mathscr{D}$ indicates the end of a cluster. The lower part shows the corresponding appearance of the single channel current (when it is assumed, if there is more than one open state, that all have the same conductance). Two clusters are shown: the first has three bursts (with three, two and two openings); the second has two bursts.

(b) *Two approaches to clusters*

(i) *Changing subscripts*

A considerable number of distributions can be found by a simple change of subscripts in the results already obtained for simple bursts in §3. There are two cases of interest, as follows.

(1) The 'per cluster' distributions. Distributions such as that of cluster length, or total open time per cluster, can be found by treating the whole cluster as though it was a single burst and ignoring any structure within the cluster. This can be achieved by neglecting the distinction between $\mathscr{B}$ and $\mathscr{C}$ states (which is, of course, like all the distinctions between subsets, to some extent arbitrary). So $\mathscr{B}$ in the results of §3 would be replaced by $\mathscr{F} = \mathscr{B} \cup \mathscr{C}$, and $\mathscr{C}$ in the results of §3 would be replaced by $\mathscr{D}$.

In summary

$$\left.\begin{aligned} &\mathscr{B} \to \mathscr{F}, \\ &\mathscr{C} \to \mathscr{D}; \\ \text{so} \quad &\mathscr{E} \to \mathscr{G} \quad \text{and} \quad \mathscr{F} \to \mathscr{T}. \end{aligned}\right\} \tag{5.3}$$

(2) The 'per burst' distributions. Distributions such as that of the number of openings per burst, or of burst length (when they are averaged over position within the cluster) can be found by ignoring clustering. This is achieved by neglecting the distinction between $\mathscr{C}$ and $\mathscr{D}$ states and replacing $\mathscr{C}$ in the results of §3 by $\mathscr{H} = \mathscr{C} \cup \mathscr{D}$. In summary

$$\mathscr{C} \to \mathscr{H}. \tag{5.4}$$

(ii) *Definition of new transition matrices*

Results which again bear an analogy with the results for bursts in §3, can be found by definition of new transition matrices by use of the principles in §2. For example, given that we start in one of the open ($\mathscr{A}$) states, the probabilities that, after oscillation any number of times between $\mathscr{A}$ and $\mathscr{B}$ and back (see (2.6)), we eventually reach $\mathscr{C}$ (directly from $\mathscr{A}$, or via $\mathscr{B}$) will be given by the transition matrix $\boldsymbol{G}_{\mathscr{A}(\mathscr{B})\mathscr{C}}$, defined as

$$\boldsymbol{G}_{\mathscr{A}(\mathscr{B})\mathscr{C}} = (\boldsymbol{I} - \boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{-1}(\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{C}} + \boldsymbol{G}_{\mathscr{A}\mathscr{C}}). \tag{5.5}$$

This is a 'burst transition matrix' that describes the transitions from the start of a burst to its end (including any silent period in $\mathscr{B}$ at the end). Similarly the gap between bursts (within

a cluster) may have (see figure 2) any number of transitions from $\mathscr{C}$ to $\mathscr{B}$ and back, before eventually reaching $\mathscr{A}$, the start of the next burst; it is described by the transition matrix defined as

$$\boldsymbol{G}_{\mathscr{C}(\mathscr{B})\mathscr{A}} = (\boldsymbol{I} - \boldsymbol{G}_{\mathscr{C}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{C}})^{-1}(\boldsymbol{G}_{\mathscr{C}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}} + \boldsymbol{G}_{\mathscr{C}\mathscr{A}}). \tag{5.6}$$

Thus the transition matrix (which has already been used in (3.88)), defined as

$$\boldsymbol{Z}_{\mathscr{A}\mathscr{A}} = \boldsymbol{G}_{\mathscr{A}(\mathscr{B})\mathscr{C}}\boldsymbol{G}_{\mathscr{C}(\mathscr{B})\mathscr{A}}, \tag{5.7}$$

describes the transitions from the start of one burst through to the start of the next burst in the same cluster. It can be used in the analysis of clusters in a manner rather analogous to the way that $\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}}$ has been used to describe the transition from the start of one opening to the start of the next opening in the same burst.

(*c*) *The start and end of a cluster*

A cluster starts (see figure 2) in *one* of the open ($\mathscr{A}$) states; so we shall, as before, need the probabilities that the cluster starts (i.e. the first opening of the first burst in the cluster starts) in each $\mathscr{A}$ state, if there is more than one. These probabilities will be denoted by $\boldsymbol{\Phi}_c$, a $1 \times k_{\mathscr{A}}$ vector (the subscript c stands for cluster); they can be found simply by treating the whole cluster as though it were one burst; so the subscript changes in (5.3) are applied to the corresponding initial vector for a burst $\boldsymbol{\Phi}_b$ (given in (3.2)). The result is

$$\boldsymbol{\Phi}_c = \frac{\boldsymbol{p}_{\mathscr{D}}(\infty)(\boldsymbol{Q}_{\mathscr{D}\mathscr{F}}\boldsymbol{G}_{\mathscr{F}\mathscr{A}} + \boldsymbol{Q}_{\mathscr{D}\mathscr{A}})}{\boldsymbol{p}_{\mathscr{D}}(\infty)(\boldsymbol{Q}_{\mathscr{D}\mathscr{F}}\boldsymbol{G}_{\mathscr{F}\mathscr{A}} + \boldsymbol{Q}_{\mathscr{D}\mathscr{A}})\boldsymbol{u}_{\mathscr{A}}}. \tag{5.8}$$

Again the denominator is just the sum of the elements in the numerator, and so the probabilities in $\boldsymbol{\Phi}_c$ sum to unity. The special cases, in which $\boldsymbol{\Phi}_c$ can be simplified, are direct analogues of those given for $\boldsymbol{\Phi}_b$ in §3*a*. The same subscript changes in (3.3) give the vector describing the end of a cluster as

$$\boldsymbol{e}_c = (\boldsymbol{G}_{\mathscr{A}\mathscr{F}}\boldsymbol{G}_{\mathscr{F}\mathscr{D}} + \boldsymbol{G}_{\mathscr{A}\mathscr{D}})\boldsymbol{u}_{\mathscr{D}}. \tag{5.9}$$

The characteristics of a burst, including its initial vector, will depend, in general, on its position within a cluster. Examples are given below of distributions derived for the mth burst in a cluster with n bursts (see, for example, (5.21) and (5.66)). However, it seems unlikely, at present, that enough clusters could be observed for such distributions to be tested; in practice the distributions that are averaged over position within the cluster are likely to be more useful. When this is done, the initial vector for a *burst* can be obtained from (3.2) by the subscript change in (5.4), giving

$$\boldsymbol{\Phi}'_b = \frac{\boldsymbol{p}_{\mathscr{H}}(\infty)(\boldsymbol{Q}_{\mathscr{H}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}} + \boldsymbol{Q}_{\mathscr{H}\mathscr{A}})}{\boldsymbol{p}_{\mathscr{H}}(\infty)(\boldsymbol{Q}_{\mathscr{H}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}} + \boldsymbol{Q}_{\mathscr{H}\mathscr{A}})\boldsymbol{u}_{\mathscr{A}}}. \tag{5.10}$$

The relation between $\boldsymbol{\Phi}_c$ and $\boldsymbol{\Phi}'_b$ is given in Appendix 2 (A 2.15). Similarly the end vector for a burst becomes, from (3.3),

$$\boldsymbol{e}'_b = (\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{H}} + \boldsymbol{G}_{\mathscr{A}\mathscr{H}})\boldsymbol{u}_{\mathscr{H}}. \tag{5.11}$$

We note here, for later use, the identities (see Appendix 2)

$$(\boldsymbol{I} - \boldsymbol{G}_{\mathscr{A}\mathscr{F}}\boldsymbol{G}_{\mathscr{F}\mathscr{A}})^{-1}\boldsymbol{e}_c = \boldsymbol{u}_{\mathscr{A}}, \tag{5.12}$$

$$(\boldsymbol{I} - \boldsymbol{Z}_{\mathscr{A}\mathscr{A}})^{-1}(\boldsymbol{I} - \boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{-1}\boldsymbol{e}_c = \boldsymbol{u}_{\mathscr{A}} \tag{5.13}$$

and $$(\boldsymbol{I}-\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{-1}\boldsymbol{e}_{\mathrm{b}}' = \boldsymbol{u}_{\mathscr{A}}. \tag{5.14}$$

These results are analogues of (3.4) that are relevant to cluster analysis.

(d) The number of openings per cluster

This distribution follows directly from (3.5), by the subscript changes in (5.3). This gives the probability of w openings per cluster as

$$P(w) = \boldsymbol{\Phi}_{\mathrm{c}}(\boldsymbol{G}_{\mathscr{A}\mathscr{F}}\boldsymbol{G}_{\mathscr{F}\mathscr{A}})^{w-1}(\boldsymbol{I}-\boldsymbol{G}_{\mathscr{A}\mathscr{F}}\boldsymbol{G}_{\mathscr{F}\mathscr{A}})\boldsymbol{u}_{\mathscr{A}}, \tag{5.15}$$

with mean $$E(w) = \boldsymbol{\Phi}_{\mathrm{c}}(\boldsymbol{I}-\boldsymbol{G}_{\mathscr{A}\mathscr{F}}\boldsymbol{G}_{\mathscr{F}\mathscr{A}})^{-1}\boldsymbol{u}_{\mathscr{A}}. \tag{5.16}$$

(e) The number of bursts per cluster

The transition matrix, $\boldsymbol{Z}_{\mathscr{A}\mathscr{A}} = \boldsymbol{G}_{\mathscr{A}(\mathscr{B})\mathscr{C}}\boldsymbol{G}_{\mathscr{C}(\mathscr{B})\mathscr{A}}$, that describes the routes from the start of one burst to the start of the next has been found in (5.5)–(5.7). Thus, from the principles in §2, $\boldsymbol{Z}_{\mathscr{A}\mathscr{A}}^{n-1}$ describes transitions up to the start of the nth burst, in which there may be any number of transitions between $\mathscr{A}$ and $\mathscr{B}$ before the nth burst ends. The probability of there being n bursts in a cluster is therefore

$$P(n) = \boldsymbol{\Phi}_{\mathrm{c}}\boldsymbol{Z}_{\mathscr{A}\mathscr{A}}^{n-1}(\boldsymbol{I}-\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{-1}\boldsymbol{e}_{\mathrm{c}} \tag{5.17}$$

$$= \boldsymbol{\Phi}_{\mathrm{c}}\boldsymbol{Z}_{\mathscr{A}\mathscr{A}}^{n-1}(\boldsymbol{I}-\boldsymbol{Z}_{\mathscr{A}\mathscr{A}})\boldsymbol{u}_{\mathscr{A}}. \tag{5.18}$$

The latter form follows from (5.13), and bears a close analogy with (3.5) and (5.15). The mean number of bursts per cluster is

$$E(n) = \boldsymbol{\Phi}_{\mathrm{c}}(\boldsymbol{I}-\boldsymbol{Z}_{\mathscr{A}\mathscr{A}})^{-1}\boldsymbol{u}_{\mathscr{A}} \tag{5.19}$$

$$= \frac{\boldsymbol{p}_{\mathscr{H}}(\infty)\,(\boldsymbol{Q}_{\mathscr{H}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}}+\boldsymbol{Q}_{\mathscr{H}\mathscr{A}})\boldsymbol{u}_{\mathscr{A}}}{\boldsymbol{p}_{\mathscr{D}}(\infty)\,(\boldsymbol{Q}_{\mathscr{D}\mathscr{F}}\boldsymbol{G}_{\mathscr{F}\mathscr{A}}+\boldsymbol{Q}_{\mathscr{D}\mathscr{A}})\boldsymbol{u}_{\mathscr{A}}}. \tag{5.20}$$

The latter form is the ratio of two scalars, the rate of transition into $\mathscr{A}$ from $\mathscr{H} = \mathscr{C} \cup \mathscr{D}$, relative to that from $\mathscr{D}$.

(f) The number of openings per burst

In general, the number of openings per burst will depend on the position of the burst within the cluster. By inspection of the routes through a cluster we can, as before, write the probability of there being r openings in the mth burst in a cluster with n bursts as

$$\begin{aligned}
&P(r;\,m,\,n)\\
&= \boldsymbol{\Phi}_{\mathrm{c}}\boldsymbol{Z}_{\mathscr{A}\mathscr{A}}^{m-1}(\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{r-1}(\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{C}}+\boldsymbol{G}_{\mathscr{A}\mathscr{C}})\,\boldsymbol{G}_{\mathscr{C}(\mathscr{B})\mathscr{A}}\boldsymbol{Z}_{\mathscr{A}\mathscr{A}}^{n-m-1}(\boldsymbol{I}-\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{-1}\boldsymbol{e}_{\mathrm{c}}/P(n), \quad m < n\\
&= \boldsymbol{\Phi}_{\mathrm{c}}\boldsymbol{Z}_{\mathscr{A}\mathscr{A}}^{m-1}(\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{r-1}\boldsymbol{e}_{\mathrm{c}}/P(n), \quad m = n\\
&= \boldsymbol{\Phi}_{\mathrm{c}}\boldsymbol{Z}_{\mathscr{A}\mathscr{A}}^{m-1}(\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{r-1}(\boldsymbol{I}-\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})\,\boldsymbol{Z}_{\mathscr{A}\mathscr{A}}^{n-m}(\boldsymbol{I}-\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{-1}\boldsymbol{e}_{\mathrm{c}}/P(n), \quad m \leqslant n,
\end{aligned} \tag{5.21}$$

where $P(n)$ is a probability that a cluster contains n bursts, from (5.18). The first two forms (respectively for all bursts but the last, and for the last burst of a cluster) are, as in (3.49)–(3.50), those with the most obvious derivation. However, the last form subsumes both the preceding results. The mean is

$$E(r;\,m,\,n) = \boldsymbol{\Phi}_{\mathrm{c}}\boldsymbol{Z}_{\mathscr{A}\mathscr{A}}^{m-1}(\boldsymbol{I}-\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{-1}\boldsymbol{Z}_{\mathscr{A}\mathscr{A}}^{n-m}(\boldsymbol{I}-\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{-1}\boldsymbol{e}_{\mathrm{c}}/P(n). \tag{5.22}$$

To average over the position of the burst within the cluster we find

$$\sum_{n=1}^{\infty}\sum_{m=1}^{n} P(r;m,n)P(n) = \sum_{m=1}^{\infty}\sum_{n=m}^{\infty} P(r;m,n)P(n).$$

When correctly normalized to unit sum, this gives the overall probability of r openings per burst as

$$P(r) = \frac{\boldsymbol{\Phi}_c(\boldsymbol{I}-\boldsymbol{Z}_{\mathscr{A}\mathscr{A}})^{-1}}{\boldsymbol{\Phi}_c(\boldsymbol{I}-\boldsymbol{Z}_{\mathscr{A}\mathscr{A}})^{-1}\boldsymbol{u}_{\mathscr{A}}} (\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{r-1}(\boldsymbol{I}-\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})(\boldsymbol{I}-\boldsymbol{Z}_{\mathscr{A}\mathscr{A}})^{-1}(\boldsymbol{I}-\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{-1}\boldsymbol{e}_c. \tag{5.23}$$

This result looks much more complicated than that previously found for bursts in (3.5). However substitution from (5.13), (5.14) and (A 2.15) into (5.23) shows that the distribution can be written simply as

$$P(r) = \boldsymbol{\Phi}'_b(\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{r-1}\boldsymbol{e}'_b, \tag{5.24}$$

where $\boldsymbol{\Phi}'_b$ and $\boldsymbol{e}'_b$ were defined in (5.10) and (5.11). This is exactly analogous to (3.5), from which it could have been obtained directly with the help of (5.4). The corresponding mean number of openings per burst, which could similarly be obtained directly from (3.7), is

$$E(r) = \boldsymbol{\Phi}'_b(\boldsymbol{I}-\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{-1}\boldsymbol{u}_{\mathscr{A}}. \tag{5.25}$$

(*g*) *The cluster length*

The Laplace transform of the required p.d.f. can be written down in the same way as the equivalent result for bursts, (3.16), if we define

$$\boldsymbol{Z}^*_{\mathscr{A}\mathscr{A}}(s) = \boldsymbol{G}^*_{\mathscr{A}(\mathscr{B})\mathscr{C}}(s)\,\boldsymbol{G}^*_{\mathscr{C}(\mathscr{B})\mathscr{A}}(s), \tag{5.26}$$

in which the terms are defined as in (5.5)–(5.7) except that every transition probability matrix, $\boldsymbol{G}$, in the definitions is replaced by $\boldsymbol{G}^*(s)$. The result therefore describes the time interval from the start of one burst to the start of the next. There may be any number of bursts per cluster; so, by direct analogy with (3.16) and (5.17), the required result is

$$f^*(s) = \boldsymbol{\Phi}_c[\boldsymbol{I}-\boldsymbol{Z}^*_{\mathscr{A}\mathscr{A}}(s)]^{-1}[\boldsymbol{I}-\boldsymbol{G}^*_{\mathscr{A}\mathscr{B}}(s)\,\boldsymbol{G}^*_{\mathscr{B}\mathscr{A}}(s)]^{-1}[\boldsymbol{G}^*_{\mathscr{A}\mathscr{F}}(s)\,\boldsymbol{G}_{\mathscr{F}\mathscr{D}}+\boldsymbol{G}^*_{\mathscr{A}\mathscr{D}}(s)]\boldsymbol{u}_{\mathscr{D}}. \tag{5.27}$$

This distribution also follows directly from the corresponding burst result, (3.17), by means of the subscript changes in (5.3). Thus the p.d.f. is

$$f(t) = \boldsymbol{\Phi}_c[\boldsymbol{P}_{\mathscr{G}\mathscr{G}}(t)]_{\mathscr{A}\mathscr{A}}(-\boldsymbol{Q}_{\mathscr{A}\mathscr{A}})\boldsymbol{e}_c. \tag{5.28}$$

This involves the subsection of $\boldsymbol{P}_{\mathscr{G}\mathscr{G}}(t) = \exp(\boldsymbol{Q}_{\mathscr{G}\mathscr{G}}t)$ that corresponds to the open ($\mathscr{A}$) states. The distribution can be expressed as the sum of $k_{\mathscr{G}} = k_{\mathscr{A}}+k_{\mathscr{B}}+k_{\mathscr{C}}$ exponential terms, as described in (1.29)–(1.33) and (3.17). The mean cluster length, from (3.19) and (5.3), is

$$m = \boldsymbol{\Phi}_c(\boldsymbol{I}-\boldsymbol{G}_{\mathscr{A}\mathscr{F}}\boldsymbol{G}_{\mathscr{F}\mathscr{A}})^{-1}(-\boldsymbol{Q}^{-1}_{\mathscr{A}\mathscr{A}})(\boldsymbol{I}-\boldsymbol{Q}_{\mathscr{A}\mathscr{F}}\boldsymbol{Q}^{-1}_{\mathscr{F}\mathscr{F}}\boldsymbol{G}_{\mathscr{F}\mathscr{A}})\boldsymbol{u}_{\mathscr{A}}. \tag{5.29}$$

This is, as expected, the sum of the mean total burst time per cluster, and the mean total gap between burst time per cluster, which are derived below, in (5.75) and (5.78).

(*h*) *The total open time per cluster*

From (3.23), (3.24) and (5.3), the distribution of the total open time per cluster is

$$f(t) = \boldsymbol{\Phi}_c \exp(-\boldsymbol{V}'_{\mathscr{A}\mathscr{A}}t)(-\boldsymbol{V}'_{\mathscr{A}\mathscr{A}})\boldsymbol{u}_{\mathscr{A}}, \tag{5.30}$$

where $$V'_{\mathscr{A}\mathscr{A}} = Q_{\mathscr{A}\mathscr{A}} + Q_{\mathscr{A}\mathscr{F}} G_{\mathscr{F}\mathscr{A}}. \tag{5.31}$$

The mean open time per cluster, from (3.26) and (5.3), is

$$m = \Phi_c(-V'_{\mathscr{A}\mathscr{A}})^{-1} u_{\mathscr{A}}. \tag{5.32}$$

If there is only one open state, the distribution becomes a simple exponential with mean (see (3.31), (5.3) and (5.12))

$$m = -1/V'_{\mathscr{A}\mathscr{A}} \tag{5.33}$$

$$= (\text{mean life in } \mathscr{A})/e_c \tag{5.34}$$

$$= 1/(Q_{\mathscr{A}\mathscr{F}} G_{\mathscr{F}\mathscr{D}} + Q_{\mathscr{A}\mathscr{D}}) u_{\mathscr{D}}. \tag{5.35}$$

Thus, as for bursts, if there is only one direct route from $\mathscr{A}$ to $\mathscr{D}$, with rate α say, so that $Q_{\mathscr{A}\mathscr{D}} u_{\mathscr{D}} = \alpha$, then the total open time per cluster will be less than $1/\alpha$ to an extent dependent on the shutting rate ($\mathscr{A}$ to $\mathscr{D}$) via $\mathscr{F}$. It will equal $1/\alpha$ if shutting via $\mathscr{F}$ is impossible.

(i) The total shut time per cluster

This can be found directly from the corresponding result for bursts, (3.38), by the substitutions in (5.3). The p.d.f. for the total shut time per burst is therefore

$$f(t) = \Phi_c e_c \delta(t) + \Phi_c G_{\mathscr{A}\mathscr{F}} \exp(W_{\mathscr{F}\mathscr{F}} t)(-W_{\mathscr{F}\mathscr{F}}) G_{\mathscr{F}\mathscr{A}} u_{\mathscr{A}}, \tag{5.36}$$

where $$W_{\mathscr{F}\mathscr{F}} = Q_{\mathscr{F}\mathscr{F}} + Q_{\mathscr{F}\mathscr{A}} G_{\mathscr{A}\mathscr{F}}. \tag{5.37}$$

The interpretation of the $\delta(t)$ term in (5.36) is exactly like that discussed after (3.38). The distribution conditional on the cluster containing at least one shut period (two openings) is, from (3.39) and (3.40),

$$f'(t) = \Phi_c G_{\mathscr{A}\mathscr{F}} \exp(W_{\mathscr{F}\mathscr{F}} t)(-W_{\mathscr{F}\mathscr{F}}) G_{\mathscr{F}\mathscr{A}} u_{\mathscr{A}} / P(w \geqslant 2), \tag{5.38}$$

where, from (3.6) or (5.15) and (5.3), the probability of getting at least two openings in a cluster is

$$P(w \geqslant 2) = \Phi_c G_{\mathscr{A}\mathscr{F}} G_{\mathscr{F}\mathscr{A}} u_{\mathscr{A}}. \tag{5.39}$$

The means corresponding to (5.36) and (5.38) are

$$m = \Phi_c G_{\mathscr{A}\mathscr{F}}(-W_{\mathscr{F}\mathscr{F}}^{-1}) G_{\mathscr{F}\mathscr{A}} u_{\mathscr{A}} \tag{5.40}$$

and $$m' = m/P(w \geqslant 2) \tag{5.41}$$

respectively.

(j) The length of individual openings

(i) *The vth opening is a cluster with w openings*

The p.d.f. is the direct analogue of the results for bursts, (3.52), found from (5.3) as

$$f(t) = \Phi_c (G_{\mathscr{A}\mathscr{F}} G_{\mathscr{F}\mathscr{A}})^{v-1} P_{\mathscr{A}\mathscr{A}}(t)(-Q_{\mathscr{A}\mathscr{A}})(G_{\mathscr{A}\mathscr{F}} G_{\mathscr{F}\mathscr{A}})^{w-v} e_c / P(w), \tag{5.42}$$

where $P(w)$, the probability of w openings per cluster, is given by (5.15).

The mean is

$$m = \Phi_c (G_{\mathscr{A}\mathscr{F}} G_{\mathscr{F}\mathscr{A}})^{v-1}(-Q_{\mathscr{A}\mathscr{A}}^{-1})(G_{\mathscr{A}\mathscr{F}} G_{\mathscr{F}\mathscr{A}})^{w-v} e_c / P(w). \tag{5.43}$$

(ii) *The kth opening of a burst with r openings, that is the mth burst in a cluster of n bursts*

Application of the usual principles gives the p.d.f. as

$$f(t) = \boldsymbol{\Phi}_{\mathrm{c}} \mathbf{Z}_{\mathscr{A}\mathscr{A}}^{m-1}(\mathbf{G}_{\mathscr{A}\mathscr{B}}\mathbf{G}_{\mathscr{B}\mathscr{A}})^{k-1}\mathbf{P}_{\mathscr{A}\mathscr{A}}(t)(-\mathbf{Q}_{\mathscr{A}\mathscr{A}})(\mathbf{G}_{\mathscr{A}\mathscr{B}}\mathbf{G}_{\mathscr{B}\mathscr{A}})^{r-k} \times (\mathbf{I}-\mathbf{G}_{\mathscr{A}\mathscr{B}}\mathbf{G}_{\mathscr{B}\mathscr{A}})\mathbf{Z}_{\mathscr{A}\mathscr{A}}^{n-m}(\mathbf{I}-\mathbf{G}_{\mathscr{A}\mathscr{B}}\mathbf{G}_{\mathscr{B}\mathscr{A}})^{-1}\mathbf{e}_{\mathrm{c}}/P(r;m,n)P(n), \tag{5.44}$$

where the denominator comes from (5.18) and (5.21).

(iii) *The kth opening in a burst with r openings*

If we average over position in the cluster (m, n) by summing the numerator of (5.44) as in (5.23), the result can be written, by use of (5.13), (5.14) and (A 2.15), as

$$f(t) = \frac{\boldsymbol{\Phi}_{\mathrm{c}}(\mathbf{I}-\mathbf{Z}_{\mathscr{A}\mathscr{A}})^{-1}(\mathbf{G}_{\mathscr{A}\mathscr{B}}\mathbf{G}_{\mathscr{B}\mathscr{A}})^{k-1}}{\boldsymbol{\Phi}_{\mathrm{c}}(\mathbf{I}-\mathbf{Z}_{\mathscr{A}\mathscr{A}})^{-1}\mathbf{u}_{\mathscr{A}}\cdot P(r)}\mathbf{P}_{\mathscr{A}\mathscr{A}}(t)(-\mathbf{Q}_{\mathscr{A}\mathscr{A}})(\mathbf{G}_{\mathscr{A}\mathscr{B}}\mathbf{G}_{\mathscr{B}\mathscr{A}})^{r-k}(\mathbf{I}-\mathbf{G}_{\mathscr{A}\mathscr{B}}\mathbf{G}_{\mathscr{B}\mathscr{A}})\mathbf{u}_{\mathscr{A}}$$
$$= \boldsymbol{\Phi}'_{\mathrm{b}}(\mathbf{G}_{\mathscr{A}\mathscr{B}}\mathbf{G}_{\mathscr{B}\mathscr{A}})^{k-1}\mathbf{P}_{\mathscr{A}\mathscr{A}}(t)(-\mathbf{Q}_{\mathscr{A}\mathscr{A}})(\mathbf{G}_{\mathscr{A}\mathscr{B}}\mathbf{G}_{\mathscr{B}\mathscr{A}})^{r-k}\mathbf{e}'_{\mathrm{b}}/P(r), \tag{5.45}$$

where $P(r)$ is given by (5.24). The latter form follows directly from the result for bursts, (3.52), by use of (5.4). The mean is therefore

$$m = \boldsymbol{\Phi}'_{\mathrm{b}}(\mathbf{G}_{\mathscr{A}\mathscr{B}}\mathbf{G}_{\mathscr{B}\mathscr{A}})^{k-1}(-\mathbf{Q}_{\mathscr{A}\mathscr{A}}^{-1})(\mathbf{G}_{\mathscr{A}\mathscr{B}}\mathbf{G}_{\mathscr{B}\mathscr{A}})^{r-k}\mathbf{e}'_{\mathrm{b}}/P(r). \tag{5.46}$$

(iv) *The overall distribution of open lifetime*

If the p.d.f. in (5.42) is averaged over position in the cluster, by direct analogy with (3.59), the overall p.d.f. for the length of an opening is found as

$$f(t) = \boldsymbol{\Phi}_{\mathrm{c}}(\mathbf{I}-\mathbf{G}_{\mathscr{A}\mathscr{F}}\mathbf{G}_{\mathscr{F}\mathscr{A}})^{-1}\mathbf{P}_{\mathscr{A}\mathscr{A}}(t)(-\mathbf{Q}_{\mathscr{A}\mathscr{A}})\mathbf{u}_{\mathscr{A}}/E(w), \tag{5.47}$$

where the mean number of openings per cluster, $E(w)$, is given in (5.16). The relation between $\boldsymbol{\Phi}_{\mathrm{c}}$ and $\boldsymbol{\Phi}'_{\mathrm{b}}$ is such that, as expected from (3.60), this can be written as

$$f(t) = \boldsymbol{\Phi}'_{\mathrm{b}}(\mathbf{I}-\mathbf{G}_{\mathscr{A}\mathscr{B}}\mathbf{G}_{\mathscr{B}\mathscr{A}})^{-1}\mathbf{P}_{\mathscr{A}\mathscr{A}}(t)(-\mathbf{Q}_{\mathscr{A}\mathscr{A}})\mathbf{u}_{\mathscr{A}}/E(r), \tag{5.48}$$

where $E(r)$, the mean number of openings per burst, is given by (5.25). The simplest version of the overall open time distribution is, not surprisingly, that found by using the initial vector appropriate to an individual opening. That is, from Colquhoun & Hawkes (1977), and by analogy with (3.63),

$$\boldsymbol{\Phi}'_{\mathrm{o}} = \mathbf{p}_{\mathscr{F}}(\infty)\mathbf{Q}_{\mathscr{F}\mathscr{A}}/\mathbf{p}_{\mathscr{F}}(\infty)\mathbf{Q}_{\mathscr{F}\mathscr{A}}\mathbf{u}_{\mathscr{A}}, \tag{5.49}$$

where $\mathscr{F} = \mathscr{B} \cup \mathscr{C} \cup \mathscr{D}$ is the set of all shut states. With this definition we obtain (see (A 2.19)) the third form of the p.d.f. as

$$f(t) = \boldsymbol{\Phi}'_{\mathrm{o}}\mathbf{P}_{\mathscr{A}\mathscr{A}}(t)(-\mathbf{Q}_{\mathscr{A}\mathscr{A}})\mathbf{u}_{\mathscr{A}} \tag{5.50}$$

with mean

$$m = \boldsymbol{\Phi}'_{\mathrm{o}}(-\mathbf{Q}_{\mathscr{A}\mathscr{A}}^{-1})\mathbf{u}_{\mathscr{A}}. \tag{5.51}$$

These results are exactly analogous to (3.64) and (3.65).

(k) All gaps within a cluster

(i) *The vth gap in a cluster with w openings*

The p.d.f. follows directly from the analogous result for bursts, (3.69), by the substitutions in (5.3), giving

$$f(t) = \boldsymbol{\Phi}_c(\boldsymbol{G}_{\mathscr{A}\mathscr{F}}\boldsymbol{G}_{\mathscr{F}\mathscr{A}})^{v-1}\boldsymbol{G}_{\mathscr{A}\mathscr{F}}\boldsymbol{P}_{\mathscr{F}\mathscr{F}}(t)(-\boldsymbol{Q}_{\mathscr{F}\mathscr{F}})\boldsymbol{G}_{\mathscr{F}\mathscr{A}}(\boldsymbol{G}_{\mathscr{A}\mathscr{F}}\boldsymbol{G}_{\mathscr{F}\mathscr{A}})^{w-v-1}\boldsymbol{e}_c/P(w), \quad v = 1, \ldots, w-1, \quad (5.52)$$

where $P(w)$ is given by (5.15).

(ii) *Overall distribution of gaps within a cluster*

If the p.d.f. in (5.52) is averaged over position in the cluster in a way exactly analogous to (3.74), the result is

$$f(t) = \boldsymbol{\Phi}_c(\boldsymbol{I}-\boldsymbol{G}_{\mathscr{A}\mathscr{F}}\boldsymbol{G}_{\mathscr{F}\mathscr{A}})^{-1}\boldsymbol{G}_{\mathscr{A}\mathscr{F}}\boldsymbol{P}_{\mathscr{F}\mathscr{F}}(t)(-\boldsymbol{Q}_{\mathscr{F}\mathscr{F}})\boldsymbol{G}_{\mathscr{F}\mathscr{A}}\boldsymbol{u}_{\mathscr{A}}/[E(w)-1]. \quad (5.53)$$

This is analogous to (3.75); the denominator, the mean number of gaps per cluster, is given in (5.16). The mean length of all gaps within clusters is

$$m = \boldsymbol{\Phi}_c(\boldsymbol{I}-\boldsymbol{G}_{\mathscr{A}\mathscr{F}}\boldsymbol{G}_{\mathscr{F}\mathscr{A}})^{-1}\boldsymbol{G}_{\mathscr{A}\mathscr{F}}(-\boldsymbol{Q}_{\mathscr{F}\mathscr{F}}^{-1})\boldsymbol{G}_{\mathscr{F}\mathscr{A}}\boldsymbol{u}_{\mathscr{A}}/[E(w)-1]. \quad (5.54)$$

(l) Gaps between bursts within a cluster

(i) *The mth gap between bursts in a cluster with n bursts*

The Laplace transform of the p.d.f. follows from the principles in §2 and the start of this section. It is

$$f^*(s) = \boldsymbol{\Phi}_c\boldsymbol{Z}_{\mathscr{A}\mathscr{A}}^{m-1}(\boldsymbol{I}-\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{-1}[\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}^*_{\mathscr{B}\mathscr{C}}(s)+\boldsymbol{G}_{\mathscr{A}\mathscr{C}}]\boldsymbol{G}^*_{\mathscr{C}(\mathscr{B})\mathscr{A}}(s)\boldsymbol{Z}_{\mathscr{A}\mathscr{A}}^{n-m-1} \times (\boldsymbol{I}-\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{-1}\boldsymbol{e}_c/P(n), \quad m = 1, \ldots, n-1, \quad (5.55)$$

where the probability of n bursts per cluster is given by (5.18), and $\boldsymbol{G}^*_{\mathscr{C}(\mathscr{B})\mathscr{A}}(s)$ is defined as in (5.6) except that all the $\boldsymbol{G}$ values are replaced by $\boldsymbol{G}^*(s)$.

(ii) *Overall distribution of gaps between bursts within clusters*

We average over position within the cluster, as before, by finding

$$\sum_{n=2}^{\infty}\sum_{m=1}^{n-1} f^*_{m,n}(s)\cdot P(n) = \sum_{m=1}^{\infty}\sum_{n=m+1}^{\infty} f^*_{m,n}(s)\cdot P(n), \quad (5.56)$$

where $f^*_{m,n}(s)$ is given by (5.55). This, with (5.13), gives

$$f^*(s) = \boldsymbol{\Phi}_c(\boldsymbol{I}-\boldsymbol{Z}_{\mathscr{A}\mathscr{A}})^{-1}(\boldsymbol{I}-\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{-1}[\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}^*_{\mathscr{B}\mathscr{C}}(s)+\boldsymbol{G}_{\mathscr{A}\mathscr{C}}]\boldsymbol{G}^*_{\mathscr{C}(\mathscr{B})\mathscr{A}}(s)\boldsymbol{u}_{\mathscr{A}}/[E(n)-1], \quad (5.57)$$

where $E(n)-1$, the mean number of gaps between bursts per cluster, is given by (5.19). The inverse transform of (5.57), the required p.d.f., can be shown to be

$$f(t) = \boldsymbol{\Psi}'_g[\boldsymbol{Q}_{\mathscr{A}\mathscr{F}}\boldsymbol{P}_{\mathscr{F}\mathscr{F}}(t)\boldsymbol{Q}_{\mathscr{F}\mathscr{A}}-\boldsymbol{Q}_{\mathscr{A}\mathscr{B}}\boldsymbol{P}_{\mathscr{B}\mathscr{B}}(t)\boldsymbol{Q}_{\mathscr{B}\mathscr{A}}]\boldsymbol{u}_{\mathscr{A}}, \quad (5.58)$$

where

$$\boldsymbol{\Psi}'_g = \boldsymbol{p}_{\mathscr{A}}(\infty)/\boldsymbol{p}_{\mathscr{A}}(\infty)(\boldsymbol{Q}_{\mathscr{A}\mathscr{F}}\boldsymbol{G}_{\mathscr{F}\mathscr{A}}-\boldsymbol{Q}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})\boldsymbol{u}_{\mathscr{A}}. \quad (5.59)$$

It is interesting that this result, in the form given in (5.58) and (5.59), is actually identical to the distribution of gaps between bursts derived in (3.84) and (3.85), before the subset $\mathscr{D}$ was introduced. The mean is thus given by (3.86) with (5.59).

(m) Gaps between clusters

The distribution of the gaps between clusters follows directly from the result for bursts, (3.85), by the substitution in (5.3). The result is

$$f(t) = \frac{\boldsymbol{p}_{\mathscr{A}}(\infty)\,[\boldsymbol{Q}_{\mathscr{A}\mathscr{F}}\boldsymbol{P}_{\mathscr{F}\mathscr{F}}(t)\,\boldsymbol{Q}_{\mathscr{F}\mathscr{A}} - \boldsymbol{Q}_{\mathscr{A}\mathscr{F}}\boldsymbol{P}_{\mathscr{F}\mathscr{F}}(t)\,\boldsymbol{Q}_{\mathscr{F}\mathscr{A}}]\boldsymbol{u}_{\mathscr{A}}}{\boldsymbol{p}_{\mathscr{A}}(\infty)\,(\boldsymbol{Q}_{\mathscr{A}\mathscr{F}}\boldsymbol{G}_{\mathscr{F}\mathscr{D}} + \boldsymbol{Q}_{\mathscr{A}\mathscr{D}})\boldsymbol{u}_{\mathscr{D}}}, \tag{5.60}$$

with mean

$$m = \frac{\boldsymbol{p}_{\mathscr{A}}(\infty)\,[\boldsymbol{Q}_{\mathscr{A}\mathscr{F}}(-\boldsymbol{Q}_{\mathscr{F}\mathscr{F}}^{-1})\,\boldsymbol{G}_{\mathscr{F}\mathscr{A}} - \boldsymbol{Q}_{\mathscr{A}\mathscr{F}}(-\boldsymbol{Q}_{\mathscr{F}\mathscr{F}}^{-1})\,\boldsymbol{G}_{\mathscr{F}\mathscr{A}}]\boldsymbol{u}_{\mathscr{A}}}{\boldsymbol{p}_{\mathscr{A}}(\infty)\,(\boldsymbol{Q}_{\mathscr{A}\mathscr{F}}\boldsymbol{G}_{\mathscr{F}\mathscr{D}} + \boldsymbol{Q}_{\mathscr{A}\mathscr{D}})\boldsymbol{u}_{\mathscr{D}}}. \tag{5.61}$$

(n) All shut times

Again the distribution of all shut times follows from (3.90) and (5.3), which give

$$f(t) = \frac{\boldsymbol{p}_{\mathscr{A}}(\infty)\,\boldsymbol{Q}_{\mathscr{A}\mathscr{F}}}{\boldsymbol{p}_{\mathscr{A}}(\infty)\,\boldsymbol{Q}_{\mathscr{A}\mathscr{F}}\boldsymbol{u}_{\mathscr{F}}}\,\boldsymbol{P}_{\mathscr{F}\mathscr{F}}(t)\,(-\boldsymbol{Q}_{\mathscr{F}\mathscr{F}})\,\boldsymbol{u}_{\mathscr{F}}, \tag{5.62}$$

with mean

$$m = \frac{\boldsymbol{p}_{\mathscr{A}}(\infty)\,\boldsymbol{Q}_{\mathscr{A}\mathscr{F}}}{\boldsymbol{p}_{\mathscr{A}}(\infty)\,\boldsymbol{Q}_{\mathscr{A}\mathscr{F}}\boldsymbol{u}_{\mathscr{F}}}\,(-\boldsymbol{Q}_{\mathscr{F}\mathscr{F}}^{-1})\boldsymbol{u}_{\mathscr{F}} \tag{5.63}$$

(o) The burst length

(i) *The mth burst in a cluster with n bursts*

For all bursts but the last in the cluster, the Laplace transform for the length of the mth burst is (compare with (5.21) and (3.49))

$$f^*(s) = \boldsymbol{\Phi}_{\mathrm{c}}\boldsymbol{Z}_{\mathscr{A}\mathscr{A}}^{m-1}[\boldsymbol{I} - \boldsymbol{G}^*_{\mathscr{A}\mathscr{B}}(s)\,\boldsymbol{G}^*_{\mathscr{B}\mathscr{A}}(s)]^{-1}\,[\boldsymbol{G}^*_{\mathscr{A}\mathscr{B}}(s)\,\boldsymbol{G}_{\mathscr{B}\mathscr{C}} + \boldsymbol{G}^*_{\mathscr{A}\mathscr{C}}(s)]\,\boldsymbol{G}_{\mathscr{C}(\mathscr{B})\mathscr{A}}\boldsymbol{Z}_{\mathscr{A}\mathscr{A}}^{n-m-1} \times (\boldsymbol{I} - \boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{-1}\boldsymbol{e}_{\mathrm{c}}/P(n), \quad m < n. \tag{5.64}$$

For the last burst in the cluster, which, unlike the others, may end with a direct transition to $\mathscr{D}$,

$$f^*(s) = \boldsymbol{\Phi}_{\mathrm{c}}\boldsymbol{Z}_{\mathscr{A}\mathscr{A}}^{m-1}[\boldsymbol{I} - \boldsymbol{G}^*_{\mathscr{A}\mathscr{B}}(s)\,\boldsymbol{G}^*_{\mathscr{B}\mathscr{A}}(s)]^{-1}\,[\boldsymbol{G}^*_{\mathscr{A}\mathscr{F}}(s)\,\boldsymbol{G}_{\mathscr{F}\mathscr{D}} + \boldsymbol{G}^*_{\mathscr{A}\mathscr{D}}(s)]\boldsymbol{u}_{\mathscr{D}}/P(n), \quad m = n. \tag{5.65}$$

As in the analogous case for bursts, (3.49)–(3.53), both of the above results can be subsumed, from (5.5) and (5.9), in the single result

$$f^*(s) = \boldsymbol{\Phi}_{\mathrm{c}}\boldsymbol{Z}_{\mathscr{A}\mathscr{A}}^{m-1}[\boldsymbol{I} - \boldsymbol{G}^*_{\mathscr{A}\mathscr{B}}(s)\,\boldsymbol{G}^*_{\mathscr{B}\mathscr{A}}(s)]^{-1}\,\boldsymbol{P}^*_{\mathscr{A}\mathscr{A}}(s)\,(-\boldsymbol{Q}_{\mathscr{A}\mathscr{A}})\,(\boldsymbol{I} - \boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})\,\boldsymbol{Z}_{\mathscr{A}\mathscr{A}}^{n-m} \times (\boldsymbol{I} - \boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{-1}\boldsymbol{e}_{\mathrm{c}}/P(n), \quad m \leqslant n. \tag{5.66}$$

(ii) *The overall distribution of burst length*

When (5.66) is averaged over position within the cluster, in a way analogous to (5.23), the result, when correctly normalized, becomes

$$f^*(s) = \boldsymbol{\Phi}_{\mathrm{c}}(\boldsymbol{I} - \boldsymbol{Z}_{\mathscr{A}\mathscr{A}})^{-1}\,[\boldsymbol{I} - \boldsymbol{G}^*_{\mathscr{A}\mathscr{B}}(s)\,\boldsymbol{G}^*_{\mathscr{B}\mathscr{A}}(s)]^{-1}\,\boldsymbol{P}^*_{\mathscr{A}\mathscr{A}}(s)\,(-\boldsymbol{Q}_{\mathscr{A}\mathscr{A}})\,(\boldsymbol{I} - \boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})\,\boldsymbol{u}_{\mathscr{A}}/E(n), \tag{5.67}$$

where $E(n)$, the mean number of bursts per cluster, is given by (5.19). With the help of (5.10), (5.11), (5.14) and (A 2.15), this can be written in the alternative form

$$f^*(s) = \boldsymbol{\Phi}'_{\mathrm{b}}[\boldsymbol{I} - \boldsymbol{G}^*_{\mathscr{A}\mathscr{B}}(s)\,\boldsymbol{G}^*_{\mathscr{B}\mathscr{A}}(s)]^{-1}\,\boldsymbol{P}^*_{\mathscr{A}\mathscr{A}}(s)\,(-\boldsymbol{Q}_{\mathscr{A}\mathscr{A}})\,\boldsymbol{e}'_{\mathrm{b}}. \tag{5.68}$$

This is, as expected, simply the analogue of (3.16) found by the substitution in (5.4). The inverse, the required overall burst length p.d.f. similarly follows directly from (5.68), or from (3.17) and (5.4), and is

$$f(t) = \boldsymbol{\Phi}'_{\mathrm{b}}[\boldsymbol{P}_{\mathscr{E}\mathscr{E}}(t)]_{\mathscr{A}\mathscr{A}}\,(-\boldsymbol{Q}_{\mathscr{A}\mathscr{A}})\,\boldsymbol{e}'_{\mathrm{b}}. \tag{5.69}$$

The mean, from (3.19), is

$$m = \boldsymbol{\Phi}'_{\mathrm{b}}(\boldsymbol{I}-\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{-1}(-\boldsymbol{Q}_{\mathscr{A}\mathscr{A}}^{-1})(\boldsymbol{I}-\boldsymbol{Q}_{\mathscr{A}\mathscr{B}}\boldsymbol{Q}_{\mathscr{B}\mathscr{B}}^{-1}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})\boldsymbol{u}_{\mathscr{A}}. \tag{5.70}$$

(p) Other overall distributions of burst characteristics

A number of other characteristics of bursts, when averaged over the position of the burst within the cluster, can be found directly from the earlier results for bursts, in §3, through (5.4), i.e. by making the substitutions $\mathscr{C} \to \mathscr{H}$, and hence $\boldsymbol{\Phi}_{\mathrm{b}} \to \boldsymbol{\Phi}'_{\mathrm{b}}$ and $\boldsymbol{e}_{\mathrm{b}} \to \boldsymbol{e}'_{\mathrm{b}}$. These include: (*a*) the length of the kth gap in a burst with r openings; (*b*) the length of the kth gap within a burst; (*c*) the overall distribution of gaps within bursts; (*d*) the total gap time per burst; (*e*) the preceding four sorts of distributions, but for open rather than gap times; (*f*) the distribution of *all* gaps between bursts, including those in $\mathscr{D}$, from (3.82)–(3.87).

(q) The total burst time per cluster

The Laplace transform of the p.d.f. of total length of time per cluster that is occupied by bursts can be found by putting $s = 0$ in those terms of (5.27) that correspond with the gaps between bursts. The result is

$$f^*(s) = \boldsymbol{\Phi}_{\mathrm{c}}[\boldsymbol{I}-\boldsymbol{Z}_1^*(s)]^{-1}[\boldsymbol{I}-\boldsymbol{G}^*_{\mathscr{A}\mathscr{B}}(s)\,\boldsymbol{G}^*_{\mathscr{B}\mathscr{A}}(s)]^{-1}\,[\boldsymbol{G}^*_{\mathscr{A}\mathscr{F}}(s)\,\boldsymbol{G}_{\mathscr{F}\mathscr{D}}+\boldsymbol{G}^*_{\mathscr{A}\mathscr{D}}(s)]\boldsymbol{u}_{\mathscr{D}}, \tag{5.71}$$

where we define the appropriate modification of (5.7) and (5.26) as

$$\boldsymbol{Z}_1^*(s) = [\boldsymbol{I}-\boldsymbol{G}^*_{\mathscr{A}\mathscr{B}}(s)\,\boldsymbol{G}^*_{\mathscr{B}\mathscr{A}}(s)]^{-1}\,[\boldsymbol{G}^*_{\mathscr{A}\mathscr{B}}(s)\,\boldsymbol{G}_{\mathscr{B}\mathscr{C}}+\boldsymbol{G}^*_{\mathscr{A}\mathscr{C}}(s)]\,\boldsymbol{G}_{\mathscr{C}(\mathscr{B})\mathscr{A}}. \tag{5.72}$$

The inverse of (5.71), which gives the required p.d.f., can be shown to be

$$f(t) = \boldsymbol{\Phi}_{\mathrm{c}}[\exp(\boldsymbol{V}_{\mathscr{E}\mathscr{E}}t)]_{\mathscr{A}\mathscr{A}}(\boldsymbol{Q}_{\mathscr{A}\mathscr{F}}\boldsymbol{G}_{\mathscr{F}\mathscr{D}}+\boldsymbol{Q}_{\mathscr{A}\mathscr{D}})\boldsymbol{u}_{\mathscr{D}}, \tag{5.73}$$

where we define

$$\boldsymbol{V}_{\mathscr{E}\mathscr{E}} = \boldsymbol{Q}_{\mathscr{E}\mathscr{E}}+\begin{bmatrix}(\boldsymbol{Q}_{\mathscr{A}\mathscr{F}}\boldsymbol{G}_{\mathscr{F}\mathscr{A}}-\boldsymbol{Q}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}}) & \boldsymbol{0}\\ \boldsymbol{0} & \boldsymbol{0}\end{bmatrix}. \tag{5.74}$$

This result bears a close analogy to the result for the total open time per burst given in (3.23)–(3.24). The mean burst time per cluster can be written as

$$m = \boldsymbol{\Phi}_{\mathrm{c}}(\boldsymbol{I}-\boldsymbol{G}_{\mathscr{A}\mathscr{F}}\boldsymbol{G}_{\mathscr{F}\mathscr{A}})^{-1}(-\boldsymbol{Q}_{\mathscr{A}\mathscr{A}}^{-1})(\boldsymbol{I}-\boldsymbol{Q}_{\mathscr{A}\mathscr{B}}\boldsymbol{Q}_{\mathscr{B}\mathscr{B}}^{-1}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})\boldsymbol{u}_{\mathscr{A}}. \tag{5.75}$$

This is, of course, the mean number of bursts per cluster, from (5.19), multiplied by the overall mean burst length, from (5.70).

(r) The total gap between burst time per cluster

As in the last section, we set $s = 0$ in those terms in (5.27) the duration of which is irrelevant, i.e. in this case the periods spent in bursts. The result is

$$f^*(s) = \boldsymbol{\Phi}_{\mathrm{c}}[\boldsymbol{I}-\boldsymbol{Z}_2^*(s)]^{-1}(\boldsymbol{I}-\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{-1}\boldsymbol{e}_{\mathrm{c}}, \tag{5.76}$$

where the appropriate variant of (5.7), (5.26) and (5.72) is defined as

$$\boldsymbol{Z}_2^*(s) = (\boldsymbol{I}-\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{-1}[\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}^*_{\mathscr{B}\mathscr{C}}(s)+\boldsymbol{G}_{\mathscr{A}\mathscr{C}}]\,[\boldsymbol{I}-\boldsymbol{G}^*_{\mathscr{C}\mathscr{B}}(s)\,\boldsymbol{G}^*_{\mathscr{B}\mathscr{C}}(s)]^{-1}$$
$$[\boldsymbol{G}^*_{\mathscr{C}\mathscr{B}}(s)\,\boldsymbol{G}^*_{\mathscr{B}\mathscr{A}}(s)+\boldsymbol{G}^*_{\mathscr{C}\mathscr{A}}(s)]. \tag{5.77}$$

The mean gap between burst time per cluster is given by

$$m = \boldsymbol{\Phi}_{\mathrm{c}}(\boldsymbol{I}-\boldsymbol{G}_{\mathscr{A}\mathscr{F}}\boldsymbol{G}_{\mathscr{F}\mathscr{A}})^{-1}(-\boldsymbol{Q}_{\mathscr{A}\mathscr{A}}^{-1})\,[\boldsymbol{Q}_{\mathscr{A}\mathscr{F}}(-\boldsymbol{Q}_{\mathscr{F}\mathscr{F}}^{-1})\,\boldsymbol{G}_{\mathscr{F}\mathscr{A}}-\boldsymbol{Q}_{\mathscr{A}\mathscr{B}}(-\boldsymbol{Q}_{\mathscr{B}\mathscr{B}}^{-1})\,\boldsymbol{G}_{\mathscr{B}\mathscr{A}}]\boldsymbol{u}_{\mathscr{A}}. \tag{5.78}$$

6. Discussion

To test experimentally any postulated mechanism of operation of ion channels, it is necessary to be able to calculate the channel behaviour that is predicted by the putative mechanism, so that this prediction can be compared with the experimental observations. For the simplest mechanisms, such as the agonist mechanism in (1.7) and the channel block mechanism in (1.9), explicit algebraic solutions can be written down fairly easily. But these mechanisms have only three kinetically distinguishable states and so they predict that not more than two components will be visible in noise and relaxation experiments, and that distributions of, for example, the duration of single channel open times, or of shut times, will have no more than two exponential components. However, it has already been observed (see, for example: Sakmann *et al.* 1980; Colquhoun & Sakmann 1981) that there are in some cases at least three components in the distribution of shut times. Mechanisms with more than three states must obviously be postulated in such cases (e.g. agonist mechanisms like (1.11), or mechanisms for desensitization and for the effect of drugs on desensitization). Such mechanisms can be tested experimentally only with the aid of results such as those given in this paper. These results provide a method for making numerical predictions of the expected behaviour of single ion channels for any mechanism, as long as the system is at equilibrium. Because the results are general, only one computer program need be written; numerical calculations can then be made for any mechanism if we specify (*a*) the appropriate set of transition rates between the various states in the mechanism (the $\boldsymbol{Q}$ matrix), and (*b*) the postulated subdivision of these states into the subsets defined at the beginning of §§1 and 5. Furthermore the only complicated parts of such a program (subroutines for finding eigenvalues and eigenvectors, and for matrix inversion) are all readily available in standard libraries.

A few examples will now be given of the ways in which the results given in this paper can be used to distinguish between various possible mechanisms.

(*a*) *Inferences from the number of components*

If bursts are visible, then there must be at least three subsets of states; so the total number of states must be at least three. Similarly, if it is possible unambiguously to distinguish clusters of bursts, there must be at least four states altogether.

Some conclusions can be drawn directly from the number of components that are found in distributions. For example the number of components in the distributions of the number of openings per burst, of open times and of total open time per burst are all predicted to be equal to the number of open states ($k_{\mathscr{A}}$). Therefore the number of open states must be at least as large as the number of components observed (which was two in the experiments of Colquhoun & Sakmann (1981) and of Cull-Candy & Parker (1982)). Of course some components might remain experimentally unresolved so that $k_{\mathscr{A}}$ could always be greater than the number of observed components. Furthermore, the conclusion depends on the assumptions (*a*) of equilibrium etc. (given at the start of the paper), (*b*) that the observations contain signals from only one sort of channel, and (*c*) that the distribution of, for example, open times is really that of single openings, i.e. that these openings do not contain short, incompletely resolved, shut periods (the distribution of the total open time per burst will obviously be a lot less sensitive to errors of this sort than is the distribution of individual open lifetimes; see Hawkes & Colquhoun 1983). Similar minimum values for the number of states in other subsets can be made from the

number of components in other burst or cluster distributions. For example, the observation by Colquhoun & Sakmann (1981) of two exponential components in the distribution of the durations of the gaps within bursts suggests that the subset $\mathscr{B}$ must contain at least two states. These two states are, of course, not necessarily those postulated in (1.11); the slower (and much smaller) component that Colquhoun & Sakmann found could, for example, represent channel block by the agonist, though the evidence mentioned below suggests that the faster component cannot be attributed to channel block.

Another example is provided by the number of components in the distribution of the burst length. Colquhoun & Sakmann (1981) were able to resolve two exponential components; so the number of burst states ($k_{\mathscr{A}}+k_{\mathscr{B}}$) must be at least two (given the assumptions listed above), The number of burst states is two for the simplest agonist mechanism (1.7), but it is easily shown (see, for example, Colquhoun & Hawkes 1981, p. 231) that no reasonable parameters can produce a fast component nearly as large as that observed. The mechanism in §4 has four burst states; and so it is also compatible with the observations, especially since the numerical calculations in §4 show that only two of the four components are predicted to be large enough to be observable.

(*b*) *Inferences from the time spent in a single state or a set of states*

The mean lifetime of a single state is simply the reciprocal of the sum of rate constants leading away from that state (i.e. the reciprocal of the appropriate diagonal element of $-\boldsymbol{Q}$). Thus anything that affects any of these rate constants will alter the lifetime. For example if there is only one open state, and addition of a drug is found to shorten the open lifetime (such that the reciprocal lifetime is linearly related to its concentration), this suggests that association of the drug with the open state causes a departure from the open state. This is what is seen with channel-blocking drugs (see, for example: Neher & Steinbach 1978; Ogden *et al.* 1981). Conversely, if the lifetime of single openings is found to be independent of the concentration of a ligand then none of the shutting routes can involve association with that ligand; for example Sakmann *et al.* (1980) found that the mean open time was independent of agonist concentration, which suggested that the bursts that they observed were not a result of ion channel block by the agonist (see also §3*a*).

More generally, the lifetime of a sojourn in any specified set of states is given by (3.63)–(3.65) (for the set $\mathscr{A}$; the same relations hold if any other subset is substituted for $\mathscr{A}$, and its complement substituted for $\mathscr{F}$). The rate constants (λ_m) for the distribution depend not only on the transition rates out of the subset (e.g. those in $\boldsymbol{Q}_{\mathscr{A}\mathscr{F}}$), but also on transition rates within the subset (e.g. in $\boldsymbol{Q}_{\mathscr{A}\mathscr{A}}$). In addition the weights, or relative areas, of the components of the distribution will depend on the initial vector, i.e. they will depend, in general (but see §3*a*), on the transition rates *into* the specified subset (e.g. in $\boldsymbol{Q}_{\mathscr{F}\mathscr{A}}$) and on the equilibrium occupancies of states not in this subset (e.g. in $\boldsymbol{p}_{\mathscr{F}}(\infty)$). The only transition rates not involved in the distribution are those between states that are *not* part of the specified subset (e.g. those in $\boldsymbol{Q}_{\mathscr{F}\mathscr{F}}$). The example in §4 has two open states (AR, A_2R) in $\mathscr{A}$. In this case the preceding generalizations show that the rate constants for the distribution of the open lifetime depend on (see (4.1) and (4.2)) the values of α_1, α_2, k^*_{+2}, x_A and k^*_{-2}. The relative area of the components depends, in addition, on β_1, β_2, k_{-2} and k_{+2} (see (4.2) and (4.18)). Anything that alters any of these will alter the distribution of open lifetimes (e.g. change in agonist concentration, effect of membrane potential on α_1 or α_2). Note that, although no channel block is involved

in this example, the mean open lifetime is expected to depend on agonist concentration to some extent. The situation is sufficiently complicated that numerical calculations may be needed to predict the nature of this dependence. For example, if the agonist concentration is increased from 0.1 to 2.5 μM for the example in §4, then $k_{+2}x_A$ is increased from 50 to 1250 s^{-1}, and the lifetime of state AR is reduced to 0.235 ms. The fast rate of the overall open time distribution is speeded up (to 4250.2 s^{-1}), but this component makes little contribution to the distribution (only 0.2% of the area), because at the higher agonist concentration an opening is almost certain to start in A_2R rather than AR ($\phi_2 = 0.997$), and so the mean open time shows only a slight increase, from 1.88 to very nearly 2 ms.

(*c*) *Inferences from the number of openings per burst*

Colquhoun & Sakmann (1981) measured bursts of openings produced by low concentrations of suberyldicholine. They found that the mean number of gaps per burst was little different whether 0.02 or 0.1 μM suberyldicholine was used. This was taken as evidence against the burst behaviour being caused by channel block by the agonist molecules; the simple channel block mechanism (1.9) would predict a fivefold increase in the mean number of gaps per burst in this experiment (see (3.15)), whereas the simple agonist mechanism, (1.7), predicts no change with concentration (the mean number of gaps per burst being β/k_{-1} in this case). However, inference is not quite so simple when more complex mechanisms are considered. The agonist mechanism (and parameter values) discussed in §4 involves no channel block, but it nevertheless (unlike the simple mechanism in (1.7)) predicts an increase in the number of gaps per burst with agonist concentration. This happens because, at high agonist concentrations, the proportion of bursts that start in A_2R rather than AR will be increased, and because of the related fact that at higher concentrations a channel in state AT will have a greater chance of rebinding a second molecule (so that the burst will probably continue), rather than losing its agonist (so that the burst ends). The magnitude of this effect is most easily checked by numerical calculation, which shows, in this case, that the expected increase in the number of gaps per burst with concentration is much less than would be predicted for a simple channel-blocking mechanism; however, the predicted increase (1.3- to 2-fold) is, if anything, greater than that observed by Colquhoun & Sakmann (1981).

(*d*) *Inferences from the total open time per burst*

The arguments leading to (3.30)–(3.33) show that measurement of the total open time per burst can be informative, particularly if there is (as in the following examples) only one open state. Such arguments have been particularly useful in the analysis of ion channel blockage (Neher & Steinbach 1978; Ogden *et al.* 1983; Neher 1983). Suppose, for example, that the open state is the last of the sequence of states, and the gap within burst states are 'proximal' to it, so that subsets are connected thus: $\mathscr{C}$–$\mathscr{B}$–$\mathscr{A}$. We denote the rate of transition out of $\mathscr{A}$ as α. Then clearly the mean length of every opening in the burst must be $1/\alpha$, and so the average total open time per burst must be greater than $1/\alpha$. This is so for the simple agonist mechanism (1.7).

On the other hand, suppose that the gap within burst states are distal to the open state, i.e. $\mathscr{C}$–$\mathscr{A}$–$\mathscr{B}$. Then the argument leading to (3.33) shows that in this case the total open time per burst must be exactly $1/\alpha$. This result was observed by Neher & Steinbach (1978), who found that the channel-blocking drug QX222 produces extended bursts of openings, but that on

average the total open time per burst was close to the mean open time ($1/\alpha$) observed in the absence of blocker. This result suggests that the blocked states are distal to the open state, and that blocked states cannot shut (i.e. reach $\mathscr{C}$) except by going through the open state. If there were a route from $\mathscr{B}$ to $\mathscr{C}$ that did not go through the open state (for example, a route via a desensitized state) then (3.32) would imply that the total open time per burst would be less than $1/\alpha$. Such an effect has been observed by Neher (1983) with higher concentrations of a channel-blocking antagonist.

Appendix 1. Some useful results for analysis of bursts

(*a*) *Miscellaneous results*

We first note two standard results which are used repeatedly; they are close analogues of the corresponding scalar results. For any matrix $\boldsymbol{H}$ with eigenvalues, λ_i, such that all $|\lambda_i| < 1$,

$$\sum_{r=0}^{\infty} \boldsymbol{H}^r = (\boldsymbol{I}-\boldsymbol{H})^{-1} \quad \text{and} \quad \sum_{r=1}^{\infty} \boldsymbol{H}^r = (\boldsymbol{I}-\boldsymbol{H})^{-1}\boldsymbol{H}, \tag{A 1.1}$$

$$\sum_{r=1}^{\infty} r\boldsymbol{H}^r = \boldsymbol{H}(\boldsymbol{I}-\boldsymbol{H})^{-2} \quad \text{and} \quad \sum_{r=1}^{\infty} r\boldsymbol{H}^{r-1} = (\boldsymbol{I}-\boldsymbol{H})^{-2}. \tag{A 1.2}$$

Next we note that, because the rows of $\boldsymbol{Q}$ sum to zero, we can obtain, from the partitioned form of $\boldsymbol{Q}$ given in (1.6),

$$\boldsymbol{Q}_{\mathscr{A}\mathscr{A}}\boldsymbol{u}_{\mathscr{A}} + \boldsymbol{Q}_{\mathscr{A}\mathscr{B}}\boldsymbol{u}_{\mathscr{B}} + \boldsymbol{Q}_{\mathscr{A}\mathscr{C}}\boldsymbol{u}_{\mathscr{C}} = 0, \tag{A 1.3}$$

$$\boldsymbol{Q}_{\mathscr{B}\mathscr{A}}\boldsymbol{u}_{\mathscr{A}} + \boldsymbol{Q}_{\mathscr{B}\mathscr{B}}\boldsymbol{u}_{\mathscr{B}} + \boldsymbol{Q}_{\mathscr{B}\mathscr{C}}\boldsymbol{u}_{\mathscr{C}} = 0, \tag{A 1.4}$$

$$\boldsymbol{Q}_{\mathscr{C}\mathscr{A}}\boldsymbol{u}_{\mathscr{A}} + \boldsymbol{Q}_{\mathscr{C}\mathscr{B}}\boldsymbol{u}_{\mathscr{B}} + \boldsymbol{Q}_{\mathscr{C}\mathscr{C}}\boldsymbol{u}_{\mathscr{C}} = 0. \tag{A 1.5}$$

If (A 1.3) is multiplied by $-\boldsymbol{Q}_{\mathscr{A}\mathscr{A}}^{-1}$, and (A 1.4) is multiplied by $-\boldsymbol{Q}_{\mathscr{B}\mathscr{B}}^{-1}$, we get, from (1.25),

$$\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{u}_{\mathscr{B}} + \boldsymbol{G}_{\mathscr{A}\mathscr{C}}\boldsymbol{u}_{\mathscr{C}} = \boldsymbol{u}_{\mathscr{A}} \tag{A 1.6}$$

and

$$\boldsymbol{G}_{\mathscr{B}\mathscr{A}}\boldsymbol{u}_{\mathscr{A}} + \boldsymbol{G}_{\mathscr{B}\mathscr{C}}\boldsymbol{u}_{\mathscr{C}} = \boldsymbol{u}_{\mathscr{B}}. \tag{A 1.7}$$

If (A 1.7) is premultiplied by $\boldsymbol{G}_{\mathscr{A}\mathscr{B}}$, and added to (A 1.6), we obtain

$$(\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{C}} + \boldsymbol{G}_{\mathscr{A}\mathscr{C}})\boldsymbol{u}_{\mathscr{C}} = (\boldsymbol{I} - \boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})\boldsymbol{u}_{\mathscr{A}}, \tag{A 1.8}$$

which proves (3.4). If (A 1.6) is premultiplied by $\boldsymbol{G}_{\mathscr{B}\mathscr{A}}$ and added to (A 1.7), we similarly obtain

$$(\boldsymbol{G}_{\mathscr{B}\mathscr{A}}\boldsymbol{G}_{\mathscr{A}\mathscr{C}} + \boldsymbol{G}_{\mathscr{B}\mathscr{C}})\boldsymbol{u}_{\mathscr{C}} = (\boldsymbol{I} - \boldsymbol{G}_{\mathscr{B}\mathscr{A}}\boldsymbol{G}_{\mathscr{A}\mathscr{B}})\boldsymbol{u}_{\mathscr{B}}. \tag{A 1.9}$$

(*b*) *The relation between* $\boldsymbol{\Phi}_0$ *and* $\boldsymbol{\Phi}_b$

It has been pointed out (§3*g*, 4.6, 4.7, 4.20) that the probabilities that any individual opening starts in a given $\mathscr{A}$ state are given by $\boldsymbol{\Phi}_0$, defined in (3.63), and that these are not the same, in general, as the probabilities that the first opening in a burst starts in a given $\mathscr{A}$ state. The latter are given by (3.2). The former were used by Colquhoun & Hawkes (1981). The relation between these initial vectors is found as follows. First we define symbols for the numerators: thus, from (3.63) we can write

$$\boldsymbol{\Phi}_0 = \boldsymbol{\Psi}_0/\boldsymbol{\Psi}_0\boldsymbol{u}_{\mathscr{A}}, \tag{A 1.10}$$

where we define, for brevity,

$$\Psi_o = p_{\mathscr{F}}(\infty)\, Q_{\mathscr{F}\mathscr{A}} = p_{\mathscr{B}}(\infty)\, Q_{\mathscr{B}\mathscr{A}} + p_{\mathscr{C}}(\infty)\, Q_{\mathscr{C}\mathscr{A}}. \tag{A 1.11}$$

Similarly, from (3.2), $$\Phi_b = \Psi_b / \Psi_b u_{\mathscr{A}}, \tag{A 1.12}$$

where $$\Psi_b = p_{\mathscr{C}}(\infty)\,(Q_{\mathscr{C}\mathscr{B}} G_{\mathscr{B}\mathscr{A}} + Q_{\mathscr{C}\mathscr{A}}). \tag{A 1.13}$$

Now note that the rate of change of state occupancy is given (Colquhoun & Hawkes 1977, equation (22)) by

$$\mathrm{d}\boldsymbol{p}(t)/\mathrm{d}t = \boldsymbol{p}(t)\, \boldsymbol{Q}; \tag{A 1.14}$$

so in the steady-state $$\boldsymbol{p}(\infty)\, \boldsymbol{Q} = \boldsymbol{0}. \tag{A 1.15}$$

When $\boldsymbol{Q}$ is partitioned as in (1.6) this relation implies that

$$p_{\mathscr{A}}(\infty)\, Q_{\mathscr{A}\mathscr{A}} + p_{\mathscr{B}}(\infty)\, Q_{\mathscr{B}\mathscr{A}} + p_{\mathscr{C}}(\infty)\, Q_{\mathscr{C}\mathscr{A}} = \boldsymbol{0}, \tag{A 1.16}$$

$$p_{\mathscr{A}}(\infty)\, Q_{\mathscr{A}\mathscr{B}} + p_{\mathscr{B}}(\infty)\, Q_{\mathscr{B}\mathscr{B}} + p_{\mathscr{C}}(\infty)\, Q_{\mathscr{C}\mathscr{B}} = \boldsymbol{0}. \tag{A 1.17}$$

Similarly, if $\boldsymbol{Q}$ is partitioned in the form

$$\boldsymbol{Q} = \begin{bmatrix} Q_{\mathscr{A}\mathscr{A}} & Q_{\mathscr{A}\mathscr{F}} \\ Q_{\mathscr{F}\mathscr{A}} & Q_{\mathscr{F}\mathscr{F}} \end{bmatrix}, \tag{A 1.18}$$

then (A 1.15) implies that

$$p_{\mathscr{A}}(\infty)\, Q_{\mathscr{A}\mathscr{A}} + p_{\mathscr{F}}(\infty)\, Q_{\mathscr{F}\mathscr{A}} = \boldsymbol{0}. \tag{A 1.19}$$

If (A 1.17) is postmultiplied by $G_{\mathscr{B}\mathscr{A}}$, and the result added to (A 1.16), we obtain

$$p_{\mathscr{A}}(\infty)\,(Q_{\mathscr{A}\mathscr{A}} + Q_{\mathscr{A}\mathscr{B}} G_{\mathscr{B}\mathscr{A}}) + p_{\mathscr{B}}(\infty)\,(Q_{\mathscr{B}\mathscr{A}} + Q_{\mathscr{B}\mathscr{B}} G_{\mathscr{B}\mathscr{A}}) + p_{\mathscr{C}}(\infty)\,(Q_{\mathscr{C}\mathscr{A}} + Q_{\mathscr{C}\mathscr{B}} G_{\mathscr{B}\mathscr{A}}) = \boldsymbol{0}. \tag{A 1.20}$$

Now, from (1.25), the central term is zero, and so, from (A 1.13), this becomes

$$p_{\mathscr{A}}(\infty)\,(Q_{\mathscr{A}\mathscr{A}} + Q_{\mathscr{A}\mathscr{B}} G_{\mathscr{B}\mathscr{A}}) + \Psi_b = \boldsymbol{0}, \tag{A 1.21}$$

so that $$p_{\mathscr{A}}(\infty)\, Q_{\mathscr{A}\mathscr{A}}(I - G_{\mathscr{A}\mathscr{B}} G_{\mathscr{B}\mathscr{A}}) + \Psi_b = \boldsymbol{0}. \tag{A 1.22}$$

Substitution for $p_{\mathscr{A}}(\infty)\, Q_{\mathscr{A}\mathscr{A}}$, from (A 1.19), gives, with (A 1.11),

$$\Psi_b = \Psi_o(I - G_{\mathscr{A}\mathscr{B}} G_{\mathscr{B}\mathscr{A}}) \tag{A 1.23}$$

and hence $$\Phi_o = \frac{\Phi_b(I - G_{\mathscr{A}\mathscr{B}} G_{\mathscr{B}\mathscr{A}})^{-1}}{\Phi_b(I - G_{\mathscr{A}\mathscr{B}} G_{\mathscr{B}\mathscr{A}})^{-1} u_{\mathscr{A}}} = \frac{\Phi_b(I - G_{\mathscr{A}\mathscr{B}} G_{\mathscr{B}\mathscr{A}})^{-1}}{E(r)}, \tag{A 1.24}$$

which is the relation required for derivation of (3.64).

(*c*) *Inversion of a partitioned matrix*

It will be useful, for example in the inversion of (3.16), to note the following standard result. We define a general partitioned matrix

$$\boldsymbol{M} = \begin{bmatrix} M_{AA} & M_{AB} \\ M_{BA} & M_{BB} \end{bmatrix} \tag{A 1.25}$$

and its inverse $$\boldsymbol{N} = \boldsymbol{M}^{-1}$$

say. As long as M_{AA} and M_{BB} are not singular, consideration of the relation $MN = I$ leads to the following form for the inverse of M:

$$N = \begin{bmatrix} N_{AA} & N_{AB} \\ N_{BA} & N_{BB} \end{bmatrix} = \begin{bmatrix} X_A & -M_{AA}^{-1} M_{AB} X_B \\ -M_{BB}^{-1} M_{BA} X_A & X_B \end{bmatrix}, \tag{A 1.26}$$

where we define

$$X_A = (I - M_{AA}^{-1} M_{AB} M_{BB}^{-1} M_{BA})^{-1} M_{AA}^{-1}, \tag{A 1.27}$$

$$X_B = (I - M_{BB}^{-1} M_{BA} M_{AA}^{-1} M_{AB})^{-1} M_{BB}^{-1}. \tag{A 1.28}$$

(i) *Application to the distribution of burst length*

First we note that the Laplace transform of this distribution, given in (3.16), can be written, from (1.21) and (3.3) as,

$$f^*(s) = \Phi_b [I - G^*_{\mathscr{A}\mathscr{B}}(s)\, G^*_{\mathscr{B}\mathscr{A}}(s)]^{-1} P^*_{\mathscr{A}\mathscr{A}}(s)\, (-Q_{\mathscr{A}\mathscr{A}})\, e_b. \tag{A 1.29}$$

Next we note that, in partitioned form, we can write

$$(sI - Q_{\mathscr{E}\mathscr{E}}) = \begin{bmatrix} sI - Q_{\mathscr{A}\mathscr{A}} & -Q_{\mathscr{A}\mathscr{B}} \\ -Q_{\mathscr{B}\mathscr{A}} & sI - Q_{\mathscr{B}\mathscr{B}} \end{bmatrix}. \tag{A 1.30}$$

The inverse of this, which is (see (1.17)) $P^*_{\mathscr{E}\mathscr{E}}(s)$, can also be written in partitioned form as

$$(sI - Q_{\mathscr{E}\mathscr{E}})^{-1} = P^*_{\mathscr{E}\mathscr{E}}(s) = \begin{bmatrix} [P^*_{\mathscr{E}\mathscr{E}}(s)]_{\mathscr{A}\mathscr{A}} & [P^*_{\mathscr{E}\mathscr{E}}(s)]_{\mathscr{A}\mathscr{B}} \\ [P^*_{\mathscr{E}\mathscr{E}}(s)]_{\mathscr{B}\mathscr{A}} & [P^*_{\mathscr{E}\mathscr{E}}(s)]_{\mathscr{B}\mathscr{B}} \end{bmatrix}. \tag{A 1.31}$$

If (A 1.30) is identified with (A 1.25), then its inverse, (A 1.31), is given by (A 1.26); so we find

$$[P^*_{\mathscr{E}\mathscr{E}}(s)]_{\mathscr{A}\mathscr{A}} = X_A = [I - G^*_{\mathscr{A}\mathscr{B}}(s)\, G^*_{\mathscr{B}\mathscr{A}}(s)]^{-1} P^*_{\mathscr{A}\mathscr{A}}(s). \tag{A 1.32}$$

Insertion of this result into (A 1.29) gives a form that inverts directly to give the p.d.f. in (3.17).

Appendix 2. Some useful results for analysis of clusters

(a) *Miscellaneous results*

Since, in (5.1), we define $\mathscr{F} = \mathscr{B} \cup \mathscr{C}$, Q can be partitioned as

$$Q = \begin{bmatrix} Q_{\mathscr{A}\mathscr{A}} & Q_{\mathscr{A}\mathscr{F}} & Q_{\mathscr{F}\mathscr{D}} \\ Q_{\mathscr{F}\mathscr{A}} & Q_{\mathscr{F}\mathscr{F}} & Q_{\mathscr{F}\mathscr{D}} \\ Q_{\mathscr{D}\mathscr{A}} & Q_{\mathscr{D}\mathscr{F}} & Q_{\mathscr{D}\mathscr{D}} \end{bmatrix}. \tag{A 2.1}$$

Proceeding by analogy with (A 1.3)–(A 1.7) we find, from the fact that the row sums of Q are zero,

$$G_{\mathscr{A}\mathscr{F}} u_{\mathscr{F}} + G_{\mathscr{A}\mathscr{D}} u_{\mathscr{D}} = u_{\mathscr{A}}, \tag{A 2.2}$$

$$G_{\mathscr{F}\mathscr{A}} u_{\mathscr{A}} + G_{\mathscr{F}\mathscr{D}} u_{\mathscr{D}} = u_{\mathscr{F}}. \tag{A 2.3}$$

If (A 2.3) is premultiplied by $G_{\mathscr{A}\mathscr{F}}$, and added to (A 2.2), we find

$$e_c = (G_{\mathscr{A}\mathscr{F}} G_{\mathscr{F}\mathscr{D}} + G_{\mathscr{A}\mathscr{D}}) u_{\mathscr{D}} = (I - G_{\mathscr{A}\mathscr{F}} G_{\mathscr{F}\mathscr{A}}) u_{\mathscr{A}}, \tag{A 2.4}$$

which proves (5.12).

Similar treatment of Q partitioned in the form

$$Q = \begin{bmatrix} Q_{\mathscr{A}\mathscr{A}} & Q_{\mathscr{A}\mathscr{B}} & Q_{\mathscr{A}\mathscr{H}} \\ Q_{\mathscr{B}\mathscr{A}} & Q_{\mathscr{B}\mathscr{B}} & Q_{\mathscr{B}\mathscr{H}} \\ Q_{\mathscr{H}\mathscr{A}} & Q_{\mathscr{H}\mathscr{B}} & Q_{\mathscr{H}\mathscr{H}} \end{bmatrix} \tag{A 2.5}$$

shows that $$e'_{\mathrm{b}} = (G_{\mathscr{A}\mathscr{B}} G_{\mathscr{B}\mathscr{H}} + G_{\mathscr{A}\mathscr{H}})\, u_{\mathscr{H}} = (I - G_{\mathscr{A}\mathscr{B}} G_{\mathscr{B}\mathscr{A}})\, u_{\mathscr{A}}, \tag{A 2.6}$$

as stated in (5.14).

To express results that involve $\mathscr{F}$ in terms of $\mathscr{B}$ and $\mathscr{C}$, we need to obtain an expression for $Q^{-1}_{\mathscr{F}\mathscr{F}}$. The general method in (A 1.25)–(A 1.28) can be applied to the partitioned form of $Q_{\mathscr{F}\mathscr{F}}$ to give

$$Q^{-1}_{\mathscr{F}\mathscr{F}} = \begin{bmatrix} Q_{\mathscr{B}\mathscr{B}} & Q_{\mathscr{B}\mathscr{C}} \\ Q_{\mathscr{C}\mathscr{B}} & Q_{\mathscr{C}\mathscr{C}} \end{bmatrix}^{-1} = \begin{bmatrix} (I - G_{\mathscr{B}\mathscr{C}} G_{\mathscr{C}\mathscr{B}})^{-1} Q^{-1}_{\mathscr{B}\mathscr{B}} & G_{\mathscr{B}\mathscr{C}} (I - G_{\mathscr{C}\mathscr{B}} G_{\mathscr{B}\mathscr{C}})^{-1} Q^{-1}_{\mathscr{C}\mathscr{C}} \\ G_{\mathscr{C}\mathscr{B}} (I - G_{\mathscr{B}\mathscr{C}} G_{\mathscr{C}\mathscr{B}})^{-1} Q^{-1}_{\mathscr{B}\mathscr{B}} & (I - G_{\mathscr{C}\mathscr{B}} G_{\mathscr{B}\mathscr{C}})^{-1} Q^{-1}_{\mathscr{C}\mathscr{C}} \end{bmatrix}. \tag{A 2.7}$$

Also, in partitioned form, we can write

$$G_{\mathscr{A}\mathscr{F}} = [G_{\mathscr{A}\mathscr{B}} \quad G_{\mathscr{A}\mathscr{C}}], \tag{A 2.8}$$

and

$$G_{\mathscr{F}\mathscr{A}} = -Q^{-1}_{\mathscr{F}\mathscr{F}} Q_{\mathscr{F}\mathscr{A}} = (-Q^{-1}_{\mathscr{F}\mathscr{F}}) \begin{bmatrix} Q_{\mathscr{B}\mathscr{A}} \\ Q_{\mathscr{C}\mathscr{A}} \end{bmatrix}. \tag{A 2.9}$$

Combination of (A 2.7)–(A 2.9) allows $G_{\mathscr{A}\mathscr{F}} G_{\mathscr{F}\mathscr{A}}$ to be written in terms of $\mathscr{A}$, $\mathscr{B}$ and $\mathscr{C}$ only. If this expansion is compared with that found by multiplying out the last three factors in the definition of $Z_{\mathscr{A}\mathscr{A}}$, i.e., from (5.5)–(5.7),

$$(G_{\mathscr{A}\mathscr{B}} G_{\mathscr{B}\mathscr{C}} + G_{\mathscr{A}\mathscr{C}})\, (I - G_{\mathscr{C}\mathscr{B}} G_{\mathscr{B}\mathscr{C}})^{-1} (G_{\mathscr{C}\mathscr{B}} G_{\mathscr{B}\mathscr{A}} + G_{\mathscr{C}\mathscr{A}}), \tag{A 2.10}$$

then use of the identity

$$G_{\mathscr{C}\mathscr{B}} (I - G_{\mathscr{B}\mathscr{C}} G_{\mathscr{C}\mathscr{B}}) = (I - G_{\mathscr{C}\mathscr{B}} G_{\mathscr{B}\mathscr{C}})\, G_{\mathscr{C}\mathscr{B}} \tag{A 2.11}$$

shows that $$Z_{\mathscr{A}\mathscr{A}} = (I - G_{\mathscr{A}\mathscr{B}} G_{\mathscr{B}\mathscr{A}})^{-1} (G_{\mathscr{A}\mathscr{F}} G_{\mathscr{F}\mathscr{A}} - G_{\mathscr{A}\mathscr{B}} G_{\mathscr{B}\mathscr{A}}). \tag{A 2.12}$$

Thus $$(I - Z_{\mathscr{A}\mathscr{A}})^{-1} = (I - G_{\mathscr{A}\mathscr{F}} G_{\mathscr{F}\mathscr{A}})^{-1} (I - G_{\mathscr{A}\mathscr{B}} G_{\mathscr{B}\mathscr{A}}). \tag{A 2.13}$$

This result, with (5.12), proves (5.13).

(*b*) *The relation between the various initial vectors*

The steady state condition, $\boldsymbol{p}(\infty)\, Q = \mathbf{0}$ (see (A 1.15)), can be applied to Q, partitioned as in (5.2), (A 2.1) and (A 2.5) in a way exactly analogous to (A 1.16)–(A 1.19). After some manipulation it is found that this implies that

$$\boldsymbol{p}_{\mathscr{H}}(\infty)\, (Q_{\mathscr{H}\mathscr{B}} G_{\mathscr{B}\mathscr{A}} + Q_{\mathscr{H}\mathscr{A}}) = \boldsymbol{p}_{\mathscr{D}}(\infty) (Q_{\mathscr{D}\mathscr{F}} G_{\mathscr{F}\mathscr{A}} + Q_{\mathscr{D}\mathscr{A}}) (I - Z_{\mathscr{A}\mathscr{A}})^{-1}. \tag{A 2.14}$$

This result, together with the definitions of $\boldsymbol{\Phi}_{\mathrm{c}}$ and $\boldsymbol{\Phi}'_{\mathrm{b}}$ in (5.8) and (5.10), shows that

$$\boldsymbol{\Phi}'_{\mathrm{b}} = \frac{\boldsymbol{\Phi}_{\mathrm{c}} (I - Z_{\mathscr{A}\mathscr{A}})^{-1}}{\boldsymbol{\Phi}_{\mathrm{c}} (I - Z_{\mathscr{A}\mathscr{A}})^{-1} \boldsymbol{u}_{\mathscr{A}}} = \boldsymbol{\Phi}_{\mathrm{c}} (I - Z_{\mathscr{A}\mathscr{A}})^{-1} / E(n), \tag{A 2.15}$$

where $E(n)$, the mean number of bursts per cluster, was given in (5.19).

Similar application of the steady state result, $\boldsymbol{p}(\infty)\, Q = \mathbf{0}$, to Q partitioned as

$$Q = \begin{bmatrix} Q_{\mathscr{A}\mathscr{A}} & Q_{\mathscr{A}\mathscr{F}} \\ Q_{\mathscr{F}\mathscr{A}} & Q_{\mathscr{F}\mathscr{F}} \end{bmatrix} \tag{A 2.16}$$

gives $$\boldsymbol{p}_{\mathscr{A}}(\infty)\,\boldsymbol{Q}_{\mathscr{A}\mathscr{A}}+\boldsymbol{p}_{\mathscr{F}}(\infty)\,\boldsymbol{Q}_{\mathscr{F}\mathscr{A}}=\boldsymbol{0}. \tag{A 2.17}$$

This result, with the previous ones, shows that

$$\boldsymbol{p}_{\mathscr{F}}(\infty)\,\boldsymbol{Q}_{\mathscr{F}\mathscr{A}}=\boldsymbol{p}_{\mathscr{H}}(\infty)\,(\boldsymbol{Q}_{\mathscr{H}\mathscr{A}}+\boldsymbol{Q}_{\mathscr{H}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})\,(\boldsymbol{I}-\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{-1}. \tag{A 2.18}$$

Thus, from (5.10) and (5.49)

$$\boldsymbol{\Phi}'_{\mathrm{o}}=\frac{\boldsymbol{\Phi}'_{\mathrm{b}}(\boldsymbol{I}-\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{-1}}{\boldsymbol{\Phi}'_{\mathrm{b}}(\boldsymbol{I}-\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{-1}\boldsymbol{u}_{\mathscr{A}}}=\boldsymbol{\Phi}'_{\mathrm{b}}(\boldsymbol{I}-\boldsymbol{G}_{\mathscr{A}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}})^{-1}/E(r), \tag{A 2.19}$$

where $E(r)$, the overall mean number of openings per burst, is given in (5.25).

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