

Properties of single ion channel currents elicited by a pulse of agonist concentration or voltage

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Experiments are often performed to study the behaviour of a single ion channel in response to a perturbation produced by a step change ('jump') in a variable that influences its equilibrium position, for example a voltage jump or jump in agonist concentration. It is also common to apply a rectangular pulse (consisting of an on jump followed by an off jump); for example brief concentration pulses are used to mimic synaptic transmission.

Assuming a general Markov mechanism for channel dynamics, we obtain theoretical probability distributions of observable characteristics that describe the non-stationary behaviour of single ion channels which are subject to a jump, or to a pulse of finite duration. These characteristics are such things as open times, shut times, first latency, burst length and length of activation. We concentrate particularly on jumps to or from a zero level of agonist, which necessitates some modification to the usual arguments to cope with having some absorbing sets of states. Where possible, we include results which make allowance for the phenomenon of time interval omission, whereby some short intervals may be missed due to imperfect resolution of the recording method. A numerical example is studied in detail.

1. Introduction and background

(a) Introduction

Single ion channel currents are, most commonly, investigated in steady state recordings. The theoretical basis for the interpretation of steady state channel currents is now quite well established (see, for example, Colquhoun & Hawkes 1995a). There are two good reasons to be interested in ion channels that are not in the steady state.

First, from the physiological point of view, ion channels are most commonly not in a steady state. For example, a nicotinic receptor channel in a muscle fibre is exposed only very briefly to the agonist (acetylcholine) that causes it to open, and during most of the time that the channel is open the agonist concentration has decayed to essentially zero.

Second, from the point of view of learning about kinetic mechanisms, the steady state response of a channel is essentially a special case of the non-stationary response (the state achieved as $t \rightarrow \infty$). Except in the very simplest cases, it is likely to be hard to identify a kinetic mechanism from steady state data alone, and it will be impossible to predict the non-stationary (physiological) behaviour from measurements of stationary behaviour alone. This phenomenon is well exemplified by the NMDA type of glutamate receptor channel. This channel shows behaviour that is considerably more complicated than that of the muscle nicotinic channel (e.g. Gibb & Colquhoun 1992), and the steady state single channel recordings cannot predict the response of the channel to a brief pulse of agonist, of the sort that it encounters in a real synapse (Edmonds & Colquhoun 1992).

The value of macroscopic jumps is well known both for voltage-operated channels (e.g. Hodgkin & Huxley 1952) and for agonist-operated channels (e.g. Franke *et al.* 1993), but measurement of single channel characteristics after a jump is potentially even more informative (e.g. Aldrich *et al.* 1983; Edmonds & Colquhoun 1992). In many experiments, a simple step change (e.g. in membrane potential or in agonist concentration) has been used. However, in order to mimic the physiological situation, it is common now to apply a brief pulse rather than a simple step. A pulse consists

of two steps, or jumps, in succession, so the theory is a bit more complicated than for a simple step. Some of the theory for non-stationary single channel behaviour was presented by Colquhoun & Hawkes (1987), but this work had three limitations. Firstly, it dealt only with simple step changes, not pulses. Secondly, it did not deal with the special case of a step change of agonist concentration from a finite value to zero (which is very common in practice, but which involves some complications). Thirdly, it did not deal with the case of limited time resolution.

The case of a jump from a finite agonist concentration to a zero agonist concentration (e.g. at the end of a pulse) is complicated because, when the agonist concentration is zero, some states (those that are reached by binding of agonist) may, depending on the details of the model, become inaccessible. In this case some matrices which are normally invertible become singular. And it will also be the case that there will be only a finite number of openings after the concentration falls to zero; eventually all channels will close (unless spontaneous openings are possible in the absence of agonist). The techniques for dealing with this case are considered in §2 and extended to pulses in §3.

The results given here apply to any sort of jump, but much emphasis is placed on jumps to and from a condition (e.g. zero agonist concentration) in which channels are all closed. In the case of voltage-activated channels, matters are complicated by the fact there is no absolute zero on the voltage scale. Nevertheless it will often be possible to make voltage jumps to and from a membrane potential where channels are essentially always closed.

In practice, the ability to resolve brief openings and shittings is always limited. Clearly, if brief openings are missed, measurements such as the latency to the first opening may have serious errors. Furthermore, in order to use the results given here for maximum likelihood fitting, we need distributions that describe what is actually observed, rather than what would have been observed if the resolution had been perfect. The distributions relevant to single channel currents observed in response to a pulse of agonist, with exact allowance for missed events, are described in §4.

The principles and notation are those employed by Colquhoun & Hawkes (1982). An introduction is given in Colquhoun & Hawkes (1995*a, b*). The underlying system is modelled by a finite-state Markov process, $X(t)$, in continuous time; $X(t) = i$, denotes the system is in state i at time t . The rate constants for transitions between states i and j ($i \neq j$) are the elements, q_{ij} , of the transition rate matrix \mathbf{Q} , and the diagonal elements, q_{ii} , are defined so that the rows sum to zero.

(b) *The ideal case*

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

$$\mathbf{Q} = \begin{bmatrix} \mathbf{Q}_{\mathcal{A}\mathcal{A}} & \mathbf{Q}_{\mathcal{A}\mathcal{F}} \\ \mathbf{Q}_{\mathcal{F}\mathcal{A}} & \mathbf{Q}_{\mathcal{F}\mathcal{F}} \end{bmatrix}. \tag{1.1}$$

The equilibrium occupancies of the various states are contained in the row vector $\mathbf{p}(\infty)$ which satisfies the equation

$$\mathbf{p}(\infty)\mathbf{Q} = \mathbf{0}, \quad \mathbf{p}(\infty)\mathbf{u} = 1, \tag{1.2}$$

where \mathbf{u} is a column vector whose elements are all unity, so the second part of the

equation merely says that the probabilities must add up to one. Methods of solving this equation are well known (e.g. see Colquhoun & Hawkes 1995*b*).

The intervals between the points when the channel opens and closes have probability densities given by the matrix

$$\mathbf{G}(t) = \begin{bmatrix} \mathbf{0} & \mathbf{G}_{\mathcal{A}\mathcal{F}}(t) \\ \mathbf{G}_{\mathcal{F}\mathcal{A}}(t) & \mathbf{0} \end{bmatrix} = \begin{bmatrix} \mathbf{0} & \exp(\mathbf{Q}_{\mathcal{A}\mathcal{A}t})\mathbf{Q}_{\mathcal{A}\mathcal{F}} \\ \exp(\mathbf{Q}_{\mathcal{F}\mathcal{F}t})\mathbf{Q}_{\mathcal{F}\mathcal{A}} & \mathbf{0} \end{bmatrix}. \quad (1.3)$$

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

$$\mathbf{G}^*(s) = \begin{bmatrix} \mathbf{0} & \mathbf{G}_{\mathcal{A}\mathcal{F}}^*(s) \\ \mathbf{G}_{\mathcal{F}\mathcal{A}}^*(s) & \mathbf{0} \end{bmatrix}, \quad (1.4)$$

where

$$\mathbf{G}_{\mathcal{A}\mathcal{F}}^*(s) = (s\mathbf{I} - \mathbf{Q}_{\mathcal{A}\mathcal{A}})^{-1}\mathbf{Q}_{\mathcal{A}\mathcal{F}}, \quad \mathbf{G}_{\mathcal{F}\mathcal{A}}^*(s) = (s\mathbf{I} - \mathbf{Q}_{\mathcal{F}\mathcal{F}})^{-1}\mathbf{Q}_{\mathcal{F}\mathcal{A}}. \quad (1.5)$$

If we consider only what state is entered next, regardless of the length of time spent in the current state, we obtain

$$\mathbf{G} = \begin{bmatrix} \mathbf{0} & \mathbf{G}_{\mathcal{A}\mathcal{F}} \\ \mathbf{G}_{\mathcal{F}\mathcal{A}} & \mathbf{0} \end{bmatrix}, \quad (1.6)$$

where we define

$$\left. \begin{aligned} \mathbf{G}_{\mathcal{A}\mathcal{F}} &= \int_0^\infty \mathbf{G}_{\mathcal{A}\mathcal{F}}(t) dt = \mathbf{G}_{\mathcal{A}\mathcal{F}}^*(0) = (-\mathbf{Q}_{\mathcal{A}\mathcal{A}})^{-1}\mathbf{Q}_{\mathcal{A}\mathcal{F}}, \\ \mathbf{G}_{\mathcal{F}\mathcal{A}} &= \int_0^\infty \mathbf{G}_{\mathcal{F}\mathcal{A}}(t) dt = \mathbf{G}_{\mathcal{F}\mathcal{A}}^*(0) = (-\mathbf{Q}_{\mathcal{F}\mathcal{F}})^{-1}\mathbf{Q}_{\mathcal{F}\mathcal{A}}. \end{aligned} \right\} \quad (1.7)$$

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

(i) Analysis of bursts

It is frequently observed that openings seem to occur in bursts, separated by long shut periods. In order to model this, the set of shut states is divided into two sets: a set of short-lived shut states, denoted \mathcal{B} , and a set of long-lived shut states, \mathcal{C} . Thus, $\mathcal{F} = \mathcal{B} \cup \mathcal{C}$. Then the \mathbf{Q} matrix can be partitioned into three blocks,

$$\mathbf{Q} = \begin{bmatrix} \mathbf{Q}_{\mathcal{A}\mathcal{A}} & \mathbf{Q}_{\mathcal{A}\mathcal{B}} & \mathbf{Q}_{\mathcal{A}\mathcal{C}} \\ \mathbf{Q}_{\mathcal{B}\mathcal{A}} & \mathbf{Q}_{\mathcal{B}\mathcal{B}} & \mathbf{Q}_{\mathcal{B}\mathcal{C}} \\ \mathbf{Q}_{\mathcal{C}\mathcal{A}} & \mathbf{Q}_{\mathcal{C}\mathcal{B}} & \mathbf{Q}_{\mathcal{C}\mathcal{C}} \end{bmatrix}. \quad (1.8)$$

The points at which transitions between the three sets of states occur, together with the states occupied at those times, form a Markov-renewal process (the terminology is not uniform in the literature, we use it in the sense of Çinlar (1975)) with

transition densities, which generalize (1.3), given by

$$\mathbf{G}(t) = \begin{bmatrix} \mathbf{0} & \mathbf{G}_{\mathcal{A}\mathcal{B}}(t) & \mathbf{G}_{\mathcal{A}\mathcal{C}}(t) \\ \mathbf{G}_{\mathcal{B}\mathcal{A}}(t) & \mathbf{0} & \mathbf{G}_{\mathcal{B}\mathcal{C}}(t) \\ \mathbf{G}_{\mathcal{C}\mathcal{A}}(t) & \mathbf{G}_{\mathcal{C}\mathcal{B}}(t) & \mathbf{0} \end{bmatrix}, \tag{1.9}$$

where, for α and β representing any of the symbols \mathcal{A} , \mathcal{B} or \mathcal{C} ,

$$\mathbf{G}_{\alpha\beta}(t) = \exp(\mathbf{Q}_{\alpha\alpha}t)\mathbf{Q}_{\alpha\beta}, \quad \alpha \neq \beta, \tag{1.10}$$

with corresponding Laplace transform

$$\mathbf{G}_{\alpha\beta}^*(s) = (s\mathbf{I} - \mathbf{Q}_{\alpha\alpha})^{-1}\mathbf{Q}_{\alpha\beta}. \tag{1.11}$$

The ij th element of $\mathbf{G}_{\alpha\beta}(t)$ gives the probability density for staying within the set of states α for a time t and then leaving for the j th state of set β , conditional on starting in the i th state of the set α .

The transition probabilities, regardless of how long it takes for the transition to occur, are given by

$$\mathbf{G}_{\alpha\beta} = \int_0^\infty \mathbf{G}_{\alpha\beta}(t) dt = \mathbf{G}_{\alpha\beta}^*(0) = (-\mathbf{Q}_{\alpha\alpha})^{-1}\mathbf{Q}_{\alpha\beta}. \tag{1.12}$$

which generalizes (1.7).

(c) *The case of limited time resolution*

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

A number of papers have attempted to deal with this problem. In particular, the first comprehensive treatment was given by Ball & Sansom (1988), but we refer to the notation and methods of Hawkes *et al.* (1990, 1992) and Colquhoun *et al.* (1996) for obtaining the stationary distributions.

In particular, the distribution of apparent shut times depends on the important matrix function ${}^{\mathcal{F}}\mathbf{R}(u)$ whose ij th element ($i, j \in \mathcal{F}$) is

$${}^{\mathcal{F}}R_{ij}(u) = P[X(u) = j \text{ and no open time is detected over } (0, u) | X(0) = i]. \tag{1.13}$$

An exact formula for this matrix function is given in Hawkes *et al.* (1990), and asymptotic values (large u) are given in Jalali & Hawkes (1992) and Hawkes *et al.* (1992) or, more succinctly, in Colquhoun *et al.* (1996).

The precision of the asymptotic solution is such that the exact solution for ${}^{\mathcal{F}}\mathbf{R}(u)$ need be calculated only for $u \leq 2\xi$, over which range the exact solution is relatively simple. These results are usually stated in terms of a matrix ${}^{\mathcal{A}}\mathbf{R}(u)$, which is like ${}^{\mathcal{F}}\mathbf{R}(u)$, but which is involved in the distribution of apparent open times (the results for shut times being obtained by exchanging symbols in the formulae) but, for the sake of completeness, and because it is the results for shut times that we need in §4, we give the explicit formulae for ${}^{\mathcal{F}}\mathbf{R}(u)$ in an Appendix.

(i) *How exact is the model for missed events?*

It is worth considering to what extent the definition of an ‘apparent opening or shutting’ corresponds with what is actually observed. What is observed will depend on both how the experimental record is filtered and how it is analysed. For example, it is possible that the effect of filtering could cause two openings occurring in quick succession to sum and so be counted as a single event longer than the dead time when in fact both openings were shorter than the dead time. This sort of error is a particular hazard when the record is fitted with the threshold crossing method; it is much less likely to occur when time course fitting is used (see Colquhoun & Sigworth 1995). Even with the latter method, some events are encountered with an odd or ambiguous shape, such that it is impossible to be sure how best to fit them. Such events are, however, quite rare and unlikely to cause serious errors in most cases.

It is worth reiterating the desirability of not relying on some theoretical dead time of the apparatus, but imposing a fixed dead time, or resolution, on the data retrospectively (as described by Colquhoun & Sigworth (1995)). (It is unfortunate that most commercial programs have not got this capability.) Imposing a longish resolution will minimize this sort of error, but will also throw away some good data. There is also a theoretical hazard in the imposing of a fixed resolution, since this process is necessarily carried out on a filtered non-ideal recording. In principle, the imposition of a fixed resolution on such a record will give results that are not identical with those that would have been found had it been possible to carry out the same imposition on the underlying ideal record. The errors from this source have never been investigated quantitatively, but are unlikely to be large.

2. Single channels following a jump to zero agonist concentration

The usual Markov models for ion channels assume that all states communicate with each other in the sense that it is possible to reach any state from any other state, via intermediate states if necessary. However, for agonist-activated channels in the absence of any agonist, this will usually not be the case; any shut channel state that can open only by binding agonist will *never* be able to open. In these circumstances, the model is said to be *reducible*, with an *absorbing* subset of the shut states which, once entered, can never be left. Then $\mathbf{Q}_{\mathcal{F}\mathcal{F}}$ will become singular and, hence, the usual formulae (see (1.7)) for matrices such as $\mathbf{G}_{\mathcal{F}\mathcal{A}}$ are invalid. Consequently, the results given by Colquhoun & Hawkes (1987) for channels following a perturbation are mostly not usable for a jump from a finite agonist concentration to zero agonist concentration.

In this section, therefore, we discuss the behaviour of a single channel following a jump from a non-zero agonist concentration to zero concentration. The jump will be supposed to take place at time $t = 0$ and the behaviour before time 0 is irrelevant to the problem. All we need to specify is a vector of initial probabilities, $\mathbf{p}(0)$, whose i th element gives the probability of the channel being in state i at the time of the concentration jump. If, for example, it is assumed that the channel has been exposed to a constant agonist concentration for a long time before the jump, then $\mathbf{p}(0)$ may be taken to be the vector of equilibrium occupancies calculated, as in (1.2), from the \mathbf{Q} matrix, \mathbf{Q}^1 say, which applies at this agonist concentration. Following the jump, it is supposed that the channel is held at zero agonist concentration for a very long

time (effectively infinity) and the matrix of transition rates which result under this regime will be denoted, in this section, by Q .

In Colquhoun & Hawkes (1982, 1987), bursts of openings and shittings were studied by dividing the set of states into three types: the subset of open states (k_A in number) is denoted \mathcal{A} ; the set of shut states, denoted \mathcal{F} , was divided into \mathcal{B} , the set of short-lived shut states (k_B in number); and the set of long-lived shut states, denoted \mathcal{C} (k_C in number). Thus $\mathcal{F} = \mathcal{B} \cup \mathcal{C}$. We use a similar division here, but \mathcal{C} must now be defined as an *absorbing set of states*. This means that once the channel has entered the set \mathcal{C} it can never get out again (infinitely long-lived) without binding an agonist molecule, so that, at zero agonist concentration, the submatrices of transition rates $Q_{\mathcal{C}\mathcal{A}} = \mathbf{0}$ and $Q_{\mathcal{C}\mathcal{B}} = \mathbf{0}$; for example, the vacant receptor, R , will belong to \mathcal{C} (as long as the model does not permit spontaneous openings in the absence of agonist). The subset \mathcal{B} , conversely, must contain all shut states from which opening is possible (\mathcal{A} can be reached) in the absence of agonist.

In order to see any openings at all after the concentration falls to zero, the channel must either (a) already be open at $t = 0$, or (b) be in a shut state belonging to the set \mathcal{B} at $t = 0$. If the channel is shut (in \mathcal{B} or \mathcal{C}) at $t = 0$, when the agonist concentration falls to zero, then no openings will be seen after $t = 0$ if the channel happened to be in a \mathcal{C} state at $t = 0$, but further openings *may* be seen if the channel happened to be in \mathcal{B} at $t = 0$ (if one or more \mathcal{B} to \mathcal{A} transitions occur), though there will be no openings if the first transition after $t = 0$ is a \mathcal{B} to \mathcal{C} transition.

With this definition of subsets, the jump to zero concentration is followed by *at most* one burst of openings before the channel is absorbed into the set of states \mathcal{C} and, in addition to separate distributions for first latency (i.e. the time up to the first opening), and for the n th open and shut times, we shall also be interested in the overall distributions of the length of this burst, the total open time in it, the number of openings in it and the overall distribution of the lengths of all the open and shut times in the burst. We shall also consider the *total activation time*, defined as the time from $t = 0$ to the end of the last opening, which is simply the first latency plus the burst length.

(a) *The fraction of channels that fail to open after $t = 0$*

As in Colquhoun & Hawkes (1987), the fraction of channels in each of the k states at $t = 0$ are kept in the row vector $\mathbf{p}(0)$, which is partitioned into \mathcal{A} , \mathcal{B} and \mathcal{C} subsets (with k_A , k_B and k_C states, respectively). Thus, $\mathbf{p}(0) = [\mathbf{p}_A(0) \mathbf{p}_B(0) \mathbf{p}_C(0)]$.

Of channels that are shut (in $\mathcal{F} = \mathcal{B} \cup \mathcal{C}$) at $t = 0$, the probability of being in \mathcal{C} (rather than \mathcal{B}), and therefore never opening, is

$$P(\mathcal{C}|\mathcal{F}) = \mathbf{p}_C(0)\mathbf{u}_C / (\mathbf{p}_F(0)\mathbf{u}_F) = P(\mathcal{C})/P(\mathcal{F}) \tag{2.1}$$

(where \mathbf{u} is a column vector with unit elements) and the probability of being in \mathcal{B} is

$$P(\mathcal{B}|\mathcal{F}) = 1 - P(\mathcal{C}|\mathcal{F}) = \mathbf{p}_B(0)\mathbf{u}_B / (\mathbf{p}_F(0)\mathbf{u}_F) = P(\mathcal{B})/P(\mathcal{F}). \tag{2.2}$$

In order to derive distributions for channels that were shut at $t = 0$, we require the relative probabilities of being in each of the \mathcal{B} states *given* that the channel is shut; this gives an initial vector defined as

$$\phi_B(0) = \mathbf{p}_B(0) / (\mathbf{p}_F(0)\mathbf{u}_F). \tag{2.3}$$

Unlike the initial vectors used elsewhere, the elements of this do not sum to 1, but rather

$$\phi_B(0)\mathbf{u}_B = \mathbf{p}_B(0)\mathbf{u}_B / (\mathbf{p}_F(0)\mathbf{u}_F) = P(\mathcal{B}|\mathcal{F}). \tag{2.4}$$

Apart from these initial probability vectors, all other matrices or vectors in this section are derived, using the notation of Colquhoun & Hawkes (1982, 1987), from the transition rate matrix \mathbf{Q} , which is obtained at zero concentration.

Let R denote a random variable representing the number of openings in the burst. Then the fraction of all those channels that were shut at $t = 0$, which fail to open at all, $P(R = 0|\mathcal{F})$ say, is given by the fraction that were in \mathcal{C} , plus the fraction that were in \mathcal{B} , but make their next transition to \mathcal{C} (rather than \mathcal{A}) and are therefore absorbed before any openings can occur, i.e.

$$P(R = 0|\mathcal{F}) = P(\mathcal{C}|\mathcal{F}) + \phi_{\mathcal{B}}(0)\mathbf{G}_{\mathcal{B}\mathcal{C}}\mathbf{u}_{\mathcal{C}}. \tag{2.5}$$

Also, the fraction of *all* channels that fail to open is

$$P(0) = P(R = 0) = P(\mathcal{F})P(R = 0|\mathcal{F}) = \mathbf{p}_{\mathcal{C}}(0)\mathbf{u}_{\mathcal{C}} + \mathbf{p}_{\mathcal{B}}(0)\mathbf{G}_{\mathcal{B}\mathcal{C}}\mathbf{u}_{\mathcal{C}} \tag{2.6}$$

and the fraction of *all* channels that produce at least one opening is

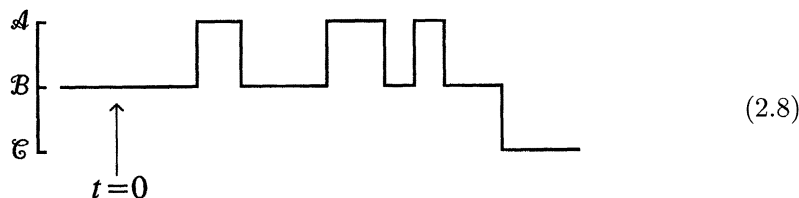
$$P(R \geq 1) = 1 - P(0) = \mathbf{p}_{\mathcal{A}}(0)\mathbf{u}_{\mathcal{A}} + \mathbf{p}_{\mathcal{B}}(0)\mathbf{G}_{\mathcal{B}\mathcal{A}}\mathbf{u}_{\mathcal{A}}. \tag{2.7}$$

In the rest of this section we will obtain various distributions (i) conditional on the channel being shut at $t = 0$, (ii) conditional on it being open at $t = 0$ and (iii) the overall distribution, regardless of the initial conditions, obtained by the appropriate combination of the previous two.

(b) *Distribution of the number of openings after $t = 0$*

(i) *Shut at $t = 0$*

The transitions between subsets, for a channel that was in \mathcal{B} at $t = 0$, could, for example, look as shown in (2.8). In this case, there are three openings followed by a final transition from \mathcal{B} to \mathcal{C} , from which it is unable to escape. Clearly, the channel must be in \mathcal{B} at $t = 0$ if it is ever to open at all.



The probability of getting r openings (for $r \geq 1$) is

$$P(r|\mathcal{F}) = \phi_{\mathcal{B}}(0)\mathbf{G}_{\mathcal{B}\mathcal{A}}(\mathbf{G}_{\mathcal{A}\mathcal{B}}\mathbf{G}_{\mathcal{B}\mathcal{A}})^{r-1}\mathbf{e}_{\mathcal{B}}, \quad r \geq 1, \tag{2.9}$$

where $\mathbf{e}_{\mathcal{B}}$ is the ‘end-of burst vector’ (Colquhoun & Hawkes 1982, equation (3.3)), defined as

$$\mathbf{e}_{\mathcal{B}} = (\mathbf{G}_{\mathcal{A}\mathcal{B}}\mathbf{G}_{\mathcal{B}\mathcal{C}} + \mathbf{G}_{\mathcal{A}\mathcal{C}})\mathbf{u}_{\mathcal{C}} = (\mathbf{I} - \mathbf{G}_{\mathcal{A}\mathcal{B}}\mathbf{G}_{\mathcal{B}\mathcal{A}})\mathbf{u}_{\mathcal{A}}, \tag{2.10}$$

whose i th element gives the probability, conditional on being in open state i at present, of absorption into \mathcal{C} and therefore never re-opening again after the current open time has finished. The cumulative form, the probability of observing some specified number, n , of openings *or more*, is

$$P(R \geq n|\mathcal{F}) = \phi_{\mathcal{B}}(0)\mathbf{G}_{\mathcal{B}\mathcal{A}}(\mathbf{G}_{\mathcal{A}\mathcal{B}}\mathbf{G}_{\mathcal{B}\mathcal{A}})^{n-1}\mathbf{u}_{\mathcal{A}}, \quad n \geq 1. \tag{2.11}$$

In particular, the probability of observing one or more openings when the channel was shut at $t = 0$ is

$$P(R \geq 1|\mathcal{F}) = \phi_B(0)\mathbf{G}_{BA}\mathbf{u}_A, \tag{2.12}$$

which, of course, equals $1 - P(0|\mathcal{F})$. This follows from (2.5), noting that $\mathbf{G}_{BC}\mathbf{u}_C + \mathbf{G}_{BA}\mathbf{u}_A = \mathbf{u}_B$ (see Colquhoun & Hawkes 1982, equation (A1.7)).

The mean number of openings (including zeros), given that the channel was shut at $t = 0$, denoted μ_0 say, is thus

$$\mu_0 = \sum_{r=0}^{\infty} rP(r|\mathcal{F}) = \sum_{n=1}^{\infty} P(R \geq n|\mathcal{F}) = \phi_B(0)\mathbf{G}_{BA}(\mathbf{I} - \mathbf{G}_{AB}\mathbf{G}_{BA})^{-1}\mathbf{u}_A. \tag{2.13}$$

If, of the channels that were shut at $t = 0$, we consider only those that produce at least one opening, the probability of there being r openings is

$$P(r|R \geq 1, \mathcal{F}) = P(r|\mathcal{F})/P(R \geq 1|\mathcal{F}), \quad r \geq 1, \tag{2.14}$$

and the mean number of openings (μ_1 say), given at least one opening, is thus

$$\mu_1 = \mu_0/P(R \geq 1|\mathcal{F}). \tag{2.15}$$

(ii) *Open at $t = 0$*

If the channel starts in \mathcal{A} , there will be at least one opening and the probability of r openings in a burst is

$$P(r|\mathcal{A}) = \phi_A(0)(\mathbf{G}_{AB}\mathbf{G}_{BA})^{r-1}\mathbf{e}_b, \quad r \geq 1, \tag{2.16}$$

where

$$\phi_A(0) = \mathbf{p}_A(0)/(\mathbf{p}_A(0)\mathbf{u}_A) = \mathbf{p}_A(0)/P(\mathcal{A})$$

gives the proportion of open channels in each state at $t = 0$. The cumulative form is

$$P(R \geq n|\mathcal{A}) = \phi_A(0)(\mathbf{G}_{AB}\mathbf{G}_{BA})^{n-1}\mathbf{u}_A, \quad n \geq 1. \tag{2.17}$$

(iii) *Overall distribution*

The overall distribution of the number of openings is obtained by combining (2.9) and (2.16) using $P(r) = P(\mathcal{F})P(r|\mathcal{F}) + P(\mathcal{A})P(r|\mathcal{A})$. Thus

$$P(r) = [\mathbf{p}_A(0) + \mathbf{p}_B(0)\mathbf{G}_{BA}](\mathbf{G}_{AB}\mathbf{G}_{BA})^{r-1}\mathbf{e}_b, \quad r \geq 1, \tag{2.18}$$

while $P(0)$ is given by (2.6).

The cumulative form, the probability of observing some specified number, n , of openings *or more*, is therefore

$$P(R \geq n) = [\mathbf{p}_A(0) + \mathbf{p}_B(0)\mathbf{G}_{BA}](\mathbf{G}_{AB}\mathbf{G}_{BA})^{n-1}\mathbf{u}_A, \quad n \geq 1, \tag{2.19}$$

while, of course, $P(R \geq 0) = 1$.

The overall mean number of openings is

$$\begin{aligned} E(R) &= \sum_{r=0}^{\infty} rP(r) = \sum_{n=1}^{\infty} P(R \geq n) \\ &= [\mathbf{p}_A(0) + \mathbf{p}_B(0)\mathbf{G}_{BA}](\mathbf{I} - \mathbf{G}_{AB}\mathbf{G}_{BA})^{-1}\mathbf{u}_A. \end{aligned} \tag{2.20}$$

Sometimes one might want to condition on having at least one opening, in which case simply divide equations (2.18)–(2.20) by $P(R \geq 1)$.

(c) *Distributions of the lengths of openings*

Below we study the probability distributions of the lengths of various open times and shut times using $f(t)$, sometimes with a subscript, to denote a probability density and $f^*(s)$ to denote its corresponding Laplace transform. In deriving these it is natural to first derive a Laplace transform, typically denoted $h^*(s)$, which not only contains information about the interval of interest but usually also contains some probability (such as the probability of a certain number of openings in a burst). The transform we actually want normally refers to a conditional distribution and is obtained by the normalization $f^*(s) = h^*(s)/h^*(0)$ so that, as usual with the Laplace transform of a probability density function, $f^*(0) = 1$ (i.e. unit total area under the PDF).

(i) *Length of the n th opening in a burst with r openings*

The distribution of the length of the n th opening in a burst with r openings is found very much as in Colquhoun & Hawkes (1982). For channels that are *shut initially* we obtain the Laplace transform. For all openings except the last we get (before normalizing)

$$h^*(s) = \phi_B(0) \mathbf{G}_{BA} (\mathbf{G}_{AB} \mathbf{G}_{BA})^{n-1} \mathbf{G}_{AB}^*(s) \mathbf{G}_{BA} (\mathbf{G}_{AB} \mathbf{G}_{BA})^{r-n-1} \mathbf{e}_b, \quad 1 \leq n < r$$

and for the last opening $r = n$

$$h^*(s) = \phi_B(0) \mathbf{G}_{BA} (\mathbf{G}_{AB} \mathbf{G}_{BA})^{n-1} [\mathbf{G}_{AB}^*(s) \mathbf{G}_{BC} + \mathbf{G}_{AC}^*(s)] \mathbf{u}_c, \quad 1 \leq n = r.$$

As in Colquhoun & Hawkes (1982), both these results can be subsumed in the single equation

$$h^*(s) = \phi_B(0) \mathbf{G}_{BA} (\mathbf{G}_{AB} \mathbf{G}_{BA})^{n-1} (s\mathbf{I} - \mathbf{Q}_{AA})^{-1} (-\mathbf{Q}_{AA}) \times (\mathbf{G}_{AB} \mathbf{G}_{BA})^{r-n} \mathbf{e}_b, \quad 1 \leq n \leq r. \quad (2.21)$$

This expression will need to be normalized by $h^*(0) = P(r|\mathcal{F})$ (see (2.9)) as described above. The PDF corresponding to $f^*(s) = h^*(s)/h^*(0)$ is thus

$$f(t) = \phi_B(0) \mathbf{G}_{BA} (\mathbf{G}_{AB} \mathbf{G}_{BA})^{n-1} \exp(\mathbf{Q}_{AA}t) (-\mathbf{Q}_{AA}) \times (\mathbf{G}_{AB} \mathbf{G}_{BA})^{r-n} \mathbf{e}_b / P(r|\mathcal{F}), \quad 1 \leq n \leq r. \quad (2.22)$$

For channels which are *open initially*, similar results can be obtained simply by replacing $\phi_B(0) \mathbf{G}_{BA}$ by $\phi_A(0)$ and $P(r|\mathcal{F})$ by $P(r|\mathcal{A})$ in (2.21)–(2.22); to get the overall distribution, regardless of initial conditions, replace them instead by $\mathbf{p}_A(0) + \mathbf{p}_B(0) \mathbf{G}_{BA}$ and $P(r)$.

(ii) *Length of the n th opening in any burst*

The distribution of the length of the n th opening in any burst for which the channel is *initially shut*, found by summing (2.21) over $r \geq n$, will be given by

$$h_n^*(s) = \phi_B(0) \mathbf{G}_{BA} (\mathbf{G}_{AB} \mathbf{G}_{BA})^{n-1} (s\mathbf{I} - \mathbf{Q}_{AA})^{-1} (-\mathbf{Q}_{AA}) \mathbf{u}_A, \quad n \geq 1. \quad (2.23)$$

This must be normalized by $h_n^*(0) = P(R \geq n|\mathcal{F})$ (see (2.11)) to give the corresponding PDF as

$$f_n(t) = \phi_B(0) \mathbf{G}_{BA} (\mathbf{G}_{AB} \mathbf{G}_{BA})^{n-1} \exp(\mathbf{Q}_{AA}t) (-\mathbf{Q}_{AA}) \mathbf{u}_A / P(R \geq n|\mathcal{F}), \quad n \geq 1. \quad (2.24)$$

For channels which are *open initially*, similar results can be obtained simply by replacing $\phi_B(0) \mathbf{G}_{BA}$ by $\phi_A(0)$ and $P(R \geq n|\mathcal{F})$ by $P(R \geq n|\mathcal{A})$ in (2.23)–(2.24);

to get the overall distribution, regardless of initial conditions, replace them instead by $\mathbf{p}_A(0) + \mathbf{p}_B(0)\mathbf{G}_{BA}$ and $P(R \geq n)$. In particular, for the overall distribution, the Laplace transform in (2.23) becomes

$$h_n^*(s) = [\mathbf{p}_A(0) + \mathbf{p}_B(0)\mathbf{G}_{BA}](\mathbf{G}_{AB}\mathbf{G}_{BA})^{n-1}(s\mathbf{I} - \mathbf{Q}_{AA})^{-1}(-\mathbf{Q}_{AA})\mathbf{u}_A, \quad n \geq 1. \tag{2.25}$$

(iii) Overall distribution of open times

If we consider the distribution of all open times, regardless of position or initial conditions, then we simply sum the functions in (2.25), giving

$$h^*(s) = \sum_{n=1}^{\infty} h_n^*(s) = [\mathbf{p}_A(0) + \mathbf{p}_B(0)\mathbf{G}_{BA}](\mathbf{I} - \mathbf{G}_{AB}\mathbf{G}_{BA})^{-1}(s\mathbf{I} - \mathbf{Q}_{AA})^{-1}(-\mathbf{Q}_{AA})\mathbf{u}_A. \tag{2.26}$$

To normalize, we divide by

$$h^*(0) = [\mathbf{p}_A(0) + \mathbf{p}_B(0)\mathbf{G}_{BA}](\mathbf{I} - \mathbf{G}_{AB}\mathbf{G}_{BA})^{-1}\mathbf{u}_A = E(R),$$

which is, from (2.20), the mean number of openings per burst. Thus, the final PDF for all open times after $t = 0$ is

$$f(t) = [\mathbf{p}_A(0) + \mathbf{p}_B(0)\mathbf{G}_{BA}](\mathbf{I} - \mathbf{G}_{AB}\mathbf{G}_{BA})^{-1} \exp(\mathbf{Q}_{AA}t)(-\mathbf{Q}_{AA})\mathbf{u}_A/E(R). \tag{2.27}$$

From the spectral expansion of the matrix $\exp(\mathbf{Q}_{AA}t)$, we see that all of these distributions are mixtures of exponentials whose time constants are the reciprocals of the k_A eigenvalues of $-\mathbf{Q}_{AA}$; the areas attached to each component will differ according to the various vectors by which $\exp(\mathbf{Q}_{AA}t)$ is pre- and post-multiplied in the above expressions.

(iv) Mean open times

The mean open time for any of these distributions is obtained simply by replacing the factor $\exp(\mathbf{Q}_{AA}t)(-\mathbf{Q}_{AA})$ by $(-\mathbf{Q}_{AA})^{-1}$ in the corresponding density function (2.22), (2.24) or (2.27). For example, from (2.27), the mean overall open time is

$$m = [\mathbf{p}_A(0) + \mathbf{p}_B(0)\mathbf{G}_{BA}](\mathbf{I} - \mathbf{G}_{AB}\mathbf{G}_{BA})^{-1}(-\mathbf{Q}_{AA})^{-1}\mathbf{u}_A/E(R). \tag{2.28}$$

(d) Distributions of the lengths of shut times

There will, of course, be no shut times to measure unless there is at least one opening. In equilibrium records, a burst with r openings will have $r - 1$ gaps-within-burst to be measured. The same will be true for a burst following a jump to zero concentration if we define the *burst* as starting at the beginning of the first open time and finishing at the end of the last opening before absorption into C . When the channel is *shut* at $t = 0$, the burst will be preceded by a shut period known as the *latency to the first opening*. When the channel is *open* at $t = 0$, the latency will be taken to be zero.

(i) Length of the n th shut time in a burst with r openings

When the channel is shut at $t = 0$, the Laplace transform of this distribution is

$$h^*(s) = \phi_B(0)(\mathbf{G}_{BA}\mathbf{G}_{AB})^n \mathbf{G}_{BA}^*(s)(\mathbf{G}_{AB}\mathbf{G}_{BA})^{r-n-1} \mathbf{e}_b$$

or, more conveniently,

$$h^*(s) = \phi_B(0)\mathbf{G}_{BA}(\mathbf{G}_{AB}\mathbf{G}_{BA})^{n-1} \mathbf{G}_{AB}\mathbf{G}_{BA}^*(s)(\mathbf{G}_{AB}\mathbf{G}_{BA})^{r-n-1} \mathbf{e}_b, \tag{2.29}$$

$$1 \leq n \leq r - 1, \quad r \geq 2.$$

This must be normalized with (see (2.9)) $h^*(0) = P(r|\mathcal{F})$, so the corresponding PDF is

$$f(t) = \phi_B(0)\mathbf{G}_{BA}(\mathbf{G}_{AB}\mathbf{G}_{AB})^{n-1}\mathbf{G}_{AB} \exp(\mathbf{Q}_{BB}t)\mathbf{Q}_{BA}(\mathbf{G}_{AB}\mathbf{G}_{BA})^{r-n-1}\mathbf{e}_b/P(r|\mathcal{F}). \tag{2.30}$$

For channels which are open initially, similar results can be obtained simply by replacing $\phi_B(0)\mathbf{G}_{BA}$ by $\phi_A(0)$ and $P(r|\mathcal{F})$ by $P(r|\mathcal{A})$ in (2.29)–(2.30); to get the overall distribution, regardless of initial conditions, replace them instead by $\mathbf{p}_A(0) + \mathbf{p}_B(0)\mathbf{G}_{BA}$ and $P(r)$.

(ii) *Length of the n th shut time in any burst*

The Laplace transform of the distribution of the n th gap in any burst, assuming the channel is *shut* at $t = 0$, can now be found by summing (2.29) over $r \geq n + 1$. This gives

$$h_n^*(s) = \phi_B(0)\mathbf{G}_{BA}(\mathbf{G}_{AB}\mathbf{G}_{BA})^{n-1}\mathbf{G}_{AB}\mathbf{G}_{BA}^*(s)\mathbf{u}_A, \quad n \geq 1, \tag{2.31}$$

which, on normalization by $h^*(0) = P(R \geq n + 1|\mathcal{F})$ (see (2.11)), gives the corresponding PDF as

$$f_n(t) = \phi_B(0)\mathbf{G}_{BA}(\mathbf{G}_{AB}\mathbf{G}_{BA})^{n-1}\mathbf{G}_{AB} \exp(\mathbf{Q}_{BB}t)\mathbf{Q}_{BA}\mathbf{u}_A/P(R \geq n + 1|\mathcal{F}), \quad n \geq 1. \tag{2.32}$$

For channels which are *open initially*, similar results can be obtained simply by replacing $\phi_B(0)\mathbf{G}_{BA}$ by $\phi_A(0)$ and $P(R \geq n + 1|\mathcal{F})$ by $P(R \geq n + 1|\mathcal{A})$ in (2.31)–(2.32); to get the overall distribution, regardless of initial conditions, replace them instead by $\mathbf{p}_A(0) + \mathbf{p}_B(0)\mathbf{G}_{BA}$ and $P(R \geq n + 1)$. In particular, for the overall distribution, the Laplace transform in (2.31) becomes

$$h_n^*(s) = [\mathbf{p}(0) + \mathbf{p}_B(0)\mathbf{G}_{BA}](\mathbf{G}_{AB}\mathbf{G}_{BA})^{n-1}\mathbf{G}_{AB}\mathbf{G}_{BA}^*(s)\mathbf{u}_A, \quad n \geq 1. \tag{2.33}$$

(iii) *Overall distribution of shut times within a burst*

If we consider the distribution of all shut times within a burst (which does not include the first latency), regardless of position or initial conditions, then we simply sum the functions in (2.33), giving

$$h^*(s) = \sum_{n=1}^{\infty} h_n^*(s) = [\mathbf{p}_A(0) + \mathbf{p}_B(0)\mathbf{G}_{BA}](\mathbf{I} - \mathbf{G}_{AB}\mathbf{G}_{BA})^{-1}\mathbf{G}_{AB}\mathbf{G}_{BA}^*(s)\mathbf{u}_A. \tag{2.34}$$

To normalize, we divide by

$$\begin{aligned} h^*(0) &= [\mathbf{p}_A(0) + \mathbf{p}_B(0)\mathbf{G}_{BA}](\mathbf{I} - \mathbf{G}_{AB}\mathbf{G}_{BA})^{-1}\mathbf{G}_{AB}\mathbf{G}_{BA}\mathbf{u}_A \\ &= [\mathbf{p}_A(0) + \mathbf{p}_B(0)\mathbf{G}_{BA}](\mathbf{I} - \mathbf{G}_{AB}\mathbf{G}_{BA})^{-1}[\mathbf{I} - (\mathbf{I} - \mathbf{G}_{AB}\mathbf{G}_{BA})]\mathbf{u}_A \\ &= [\mathbf{p}_A(0) + \mathbf{p}_B(0)\mathbf{G}_{BA}](\mathbf{I} - \mathbf{G}_{AB}\mathbf{G}_{BA})^{-1}\mathbf{u}_A - [\mathbf{p}_A(0) + \mathbf{p}_B(0)\mathbf{G}_{BA}]\mathbf{u}_A, \end{aligned}$$

which, using (2.19) and (2.20), simplifies to

$$h^*(0) = E(R) - P(R \geq 1) = [E(R|R \geq 1) - 1]P(R \geq 1). \tag{2.35}$$

Finally, the PDF is

$$f(t) = [\mathbf{p}_A(0) + \mathbf{p}_B(0)\mathbf{G}_{BA}](\mathbf{I} - \mathbf{G}_{AB}\mathbf{G}_{BA})^{-1}\mathbf{G}_{AB} \exp(\mathbf{Q}_{BB}t)\mathbf{Q}_{BA}\mathbf{u}_A/h^*(0). \tag{2.36}$$

(iv) Mean shut times

The mean shut time for any of these distributions is obtained simply by replacing the factor $\exp(\mathbf{Q}_{BB}t)\mathbf{Q}_{BA}$ by $(-\mathbf{Q}_{BB})^{-1}\mathbf{G}_{BA}$ in the corresponding density function (2.30), (2.32) or (2.36). So, for example, from (2.36), the mean overall shut time within a burst is

$$m = [\mathbf{p}_A(0) + \mathbf{p}_B(0)\mathbf{G}_{BA}](\mathbf{I} - \mathbf{G}_{AB}\mathbf{G}_{BA})^{-1}\mathbf{G}_{AB}(-\mathbf{Q}_{BB})^{-1}\mathbf{G}_{BA}\mathbf{u}_A/h^*(0). \quad (2.37)$$

Ball *et al.* (1989) considered channel behaviour following a jump, assuming the channel was shut initially. They were mainly concerned with the mean of the n th open time and the mean of the n th shut time as functions of n but, in the process, obtained equations for the probability densities equivalent to (2.24) and (2.32).

(v) The latency to the first opening

If the channel is *shut* at $t = 0$, there will be a shut time before the start of the first opening, if any. Its distribution when preceding bursts with r openings is similar to (2.30) with $n = 0$, omitting the factor $\mathbf{G}_{BA}(\mathbf{G}_{AB}\mathbf{G}_{BA})^{r-1}\mathbf{G}_{AB}$, and has PDF

$$f(t) = \phi_B(0) \exp(\mathbf{Q}_{BB}t)\mathbf{Q}_{BA}(\mathbf{G}_{AB}\mathbf{G}_{BA})^{r-1}\mathbf{e}_b/P(r|\mathcal{F}), \quad r \geq 1. \quad (2.38)$$

Similarly, its PDF, regardless of the number of subsequent openings, is similar to putting $n = 0$ in (2.32), again omitting the factor $\mathbf{G}_{BA}(\mathbf{G}_{AB}\mathbf{G}_{BA})^{n-1}\mathbf{G}_{AB}$, and so we get the obvious result

$$f(t) = \phi_B(0) \exp(\mathbf{Q}_{BB}t)\mathbf{Q}_{BA}\mathbf{u}_A/P(R \geq 1|\mathcal{F}). \quad (2.39)$$

The latency is, of course, zero if the channel is open at $t = 0$.

As above, the mean of either of these two distributions is obtained simply by replacing the factor $\exp(\mathbf{Q}_{BB}t)\mathbf{Q}_{BA}$ by $(-\mathbf{Q}_{BB})^{-1}\mathbf{G}_{BA}$ in the corresponding density function.

From the spectral expansion of the matrix $\exp(\mathbf{Q}_{BB}t)$, we see that all of these distributions of shut times are mixtures of exponentials whose time constants are the reciprocals of the k_B eigenvalues of $-\mathbf{Q}_{BB}$; the areas attached to each component will differ according to the various vectors by which $\exp(\mathbf{Q}_{BB}t)$ is pre- and post-multiplied in the above expressions.

(e) The length of the burst

As discussed above, we define the *burst* as starting at the beginning of the first open time and finishing at the end of the last opening before absorption into \mathcal{C} . For a channel which is *shut* at $t = 0$, we ignore the first latency and note that the result is the same as the conventional burst length PDF (Colquhoun & Hawkes 1982, equation (3.16)), except that the initial vector is taken as $\phi_B(0)\mathbf{G}_{BA}$. Thus, the Laplace transform is given by

$$\begin{aligned} h^*(s) &= \phi_B(0)\mathbf{G}_{BA} \sum_{r=1}^{\infty} [\mathbf{G}_{AB}^*(s)\mathbf{G}_{BA}^*(s)]^{r-1} (\mathbf{G}_{AB}^*(s)\mathbf{G}_{BC} + \mathbf{G}_{AC}^*(s))\mathbf{u}_C \\ &= \phi_B(0)\mathbf{G}_{BA} [\mathbf{I} - \mathbf{G}_{AB}^*(s)\mathbf{G}_{BA}^*(s)]^{-1} (\mathbf{G}_{AB}^*(s)\mathbf{G}_{BC} + \mathbf{G}_{AC}^*(s))\mathbf{u}_C. \end{aligned} \quad (2.40)$$

This must be normalized by $h^*(0) = P(R \geq 1|\mathcal{F})$ and inverted, as in Colquhoun & Hawkes (1982), to give the PDF

$$f(t) = \phi_B(0)\mathbf{G}_{BA}[\exp(\mathbf{Q}_{EE}t)]_{AA}(-\mathbf{Q}_{AA})\mathbf{e}_b/P(R \geq 1|\mathcal{F}). \quad (2.41)$$

This is because a burst consists of a sojourn in $\mathcal{E} = \mathcal{A} \cup \mathcal{B}$, starting and ending in the open states, \mathcal{A} (see Colquhoun & Hawkes 1995a, § 13.4, for an elementary exposition). This is a mixture of exponentials whose time constants are the reciprocals of the $k_{\mathcal{E}}$ eigenvalues of $-\mathbf{Q}_{\mathcal{E}\mathcal{E}}$.

For channels which are *open* at $t = 0$, similar results can be obtained simply by replacing $\phi_{\mathcal{B}}(0)\mathbf{G}_{\mathcal{B}\mathcal{A}}$ by $\phi_{\mathcal{A}}(0)$ and $P(R \geq 1|\mathcal{F})$ by $P(R \geq 1|\mathcal{A})$ in (2.40)–(2.41); to get the overall distribution, regardless of initial conditions, replace them instead by $\mathbf{p}_{\mathcal{A}}(0) + \mathbf{p}_{\mathcal{B}}(0)\mathbf{G}_{\mathcal{B}\mathcal{A}}$ and $P(R \geq 1)$.

The mean of any of these distributions can be obtained from (2.41) by replacing $[\exp(\mathbf{Q}_{\mathcal{E}\mathcal{E}}t)]_{\mathcal{A}\mathcal{A}}(-\mathbf{Q}_{\mathcal{A}\mathcal{A}})\mathbf{e}_{\mathcal{B}}$ by $(\mathbf{I} - \mathbf{G}_{\mathcal{A}\mathcal{B}}\mathbf{G}_{\mathcal{B}\mathcal{A}})^{-1}[(-\mathbf{Q}_{\mathcal{A}\mathcal{A}})^{-1} + \mathbf{G}_{\mathcal{A}\mathcal{B}}(-\mathbf{Q}_{\mathcal{B}\mathcal{B}})^{-1}\mathbf{G}_{\mathcal{B}\mathcal{A}}]\mathbf{u}_{\mathcal{A}}$, which is mean total open time per burst plus mean total shut time per burst. More simply, the mean can also be obtained by replacing $\exp(\mathbf{Q}_{\mathcal{E}\mathcal{E}}t)$ in (2.41) by $(-\mathbf{Q}_{\mathcal{E}\mathcal{E}})^{-2}$ (not forgetting then to take the $\mathcal{A}\mathcal{A}$ submatrix of the result) and using the appropriate initial vector and normalising factor as discussed in the previous paragraph.

(f) *The length of the entire activation*

We define the *length of the activation* as the time from the moment of the concentration jump ($t = 0$) until the end of the last opening. Thus,

$$\text{length of activation} = \text{first latency} + \text{burst length.}$$

Of course, if the channel is *open* at $t = 0$, the first latency is zero and the length of activation and burst length are the same; the distributions will, therefore, be identical in these circumstances.

When the channel is *shut* at $t = 0$, the results on burst length are simply modified to include the first latency. The Laplace transform becomes

$$h^*(s) = \phi_{\mathcal{B}}(0)\mathbf{G}_{\mathcal{B}\mathcal{A}}^*(s)[\mathbf{I} - \mathbf{G}_{\mathcal{A}\mathcal{B}}^*(s)\mathbf{G}_{\mathcal{B}\mathcal{A}}^*(s)]^{-1}(\mathbf{G}_{\mathcal{A}\mathcal{B}}^*(s)\mathbf{G}_{\mathcal{B}\mathcal{C}} + \mathbf{G}_{\mathcal{A}\mathcal{C}}^*(s))\mathbf{u}_{\mathcal{C}} \quad (2.42)$$

and the corresponding normalized PDF is

$$f(t) = \phi_{\mathcal{B}}(0)[\exp(\mathbf{Q}_{\mathcal{E}\mathcal{E}}t)]_{\mathcal{B}\mathcal{A}}(-\mathbf{Q}_{\mathcal{A}\mathcal{A}})\mathbf{e}_{\mathcal{B}}/P(R \geq 1|\mathcal{F}). \quad (2.43)$$

Thus the $\mathcal{B}\mathcal{A}$ subsection, rather than the $\mathcal{A}\mathcal{A}$ subsection, of $\exp(\mathbf{Q}_{\mathcal{E}\mathcal{E}}t)$ is needed in this case.

To get the overall distribution, regardless of initial conditions, combine the two cases to give

$$h^*(s) = [\mathbf{p}_{\mathcal{A}}(0) + \mathbf{p}_{\mathcal{B}}(0)\mathbf{G}_{\mathcal{B}\mathcal{A}}^*(s)][\mathbf{I} - \mathbf{G}_{\mathcal{A}\mathcal{B}}^*(s)\mathbf{G}_{\mathcal{B}\mathcal{A}}^*(s)]^{-1}(\mathbf{G}_{\mathcal{A}\mathcal{B}}^*(s)\mathbf{G}_{\mathcal{B}\mathcal{C}} + \mathbf{G}_{\mathcal{A}\mathcal{C}}^*(s))\mathbf{u}_{\mathcal{C}}, \quad (2.44)$$

which is normalized by $h^*(0) = P(R \geq 1)$ and inverted to give

$$f(t) = \mathbf{p}_{\mathcal{E}}(0)[\exp(\mathbf{Q}_{\mathcal{E}\mathcal{E}}t)]_{\mathcal{E}\mathcal{A}}(-\mathbf{Q}_{\mathcal{A}\mathcal{A}})\mathbf{e}_{\mathcal{B}}/P(R \geq 1). \quad (2.45)$$

Again, if we use a spectral expansion, we see that all of these distributions are mixtures of exponentials whose time constants are the reciprocals of the $k_{\mathcal{E}}$ eigenvalues of $-\mathbf{Q}_{\mathcal{E}\mathcal{E}}$. Thus, the time constants are the same as those for the burst length distribution; only the relative areas of the components differ.

Clearly, from the definition preceding (2.42), we have

$$\text{mean length of activation} = \text{mean latency} + \text{mean burst length,}$$

where algebraic expressions for the two means on the right, under appropriate conditioning, are given in the two previous subsections. More directly, simply replace

$\exp(\mathbf{Q}_{\mathcal{E}\mathcal{E}}t)$ by $(-\mathbf{Q}_{\mathcal{E}\mathcal{E}})^{-2}$ in (2.43) or (2.45) (not forgetting then to take the appropriate submatrix, $\mathcal{B}\mathcal{A}$ or $\mathcal{E}\mathcal{A}$, of the result). Thus, for example, the mean overall total activation time, assuming it is non-zero, is obtained from (2.45) as

$$m = \mathbf{p}_{\mathcal{E}}(0)[(-\mathbf{Q}_{\mathcal{E}\mathcal{E}})^{-2}]_{\mathcal{E}\mathcal{A}}(-\mathbf{Q}_{\mathcal{A}\mathcal{A}})\mathbf{e}_b/P(R \geq 1). \tag{2.46}$$

(g) *The macroscopic time course*

The macroscopic time course following a jump is given by

$$I(t) = N\gamma P(\text{channel open at } t) = N\gamma \mathbf{p}(0) \exp(\mathbf{Q}t) \begin{bmatrix} \mathbf{u}_{\mathcal{A}} \\ \mathbf{0} \end{bmatrix}, \tag{2.47}$$

where γ is the conductance of the open channel and N is the number of channels. From the spectral expansion of $\exp(\mathbf{Q}t)$ it follows that, in general, the time course is a mixture of $k - 1$ exponential components whose time constants are reciprocals of the eigenvalues of $-\mathbf{Q}$, omitting the zero eigenvalue.

However, at zero concentration, this result can be simplified. In the last two sections we have partitioned the matrix \mathbf{Q} into $\mathbf{Q}_{\mathcal{E}\mathcal{E}}$, $\mathbf{Q}_{\mathcal{E}\mathcal{C}}$, $\mathbf{Q}_{\mathcal{C}\mathcal{E}}$ and $\mathbf{Q}_{\mathcal{C}\mathcal{C}}$. But, at zero agonist concentration, the set \mathcal{C} is absorbing, so $\mathbf{Q}_{\mathcal{C}\mathcal{E}} = \mathbf{0}$. Thus, \mathbf{Q} has a ‘block upper triangular’ form. This implies that the eigenvalues of \mathbf{Q} itself will be the same as the $k_{\mathcal{E}}$ eigenvalues of $\mathbf{Q}_{\mathcal{E}\mathcal{E}}$, plus the $k_{\mathcal{C}}$ eigenvalues of $\mathbf{Q}_{\mathcal{C}\mathcal{C}}$. Furthermore, it turns out that the components that have eigenvalues of $\mathbf{Q}_{\mathcal{C}\mathcal{C}}$ have zero amplitude in the macroscopic response, so the macroscopic response to a jump to zero concentration will have only $k_{\mathcal{E}}$ components with time constants that are the reciprocals of the eigenvalues of $-\mathbf{Q}_{\mathcal{E}\mathcal{E}}$, rather than the $k - 1$ components that the macroscopic jump will have in general. Thus, the macroscopic time course will have the same time constants as the distributions of burst length and of activation length (see also §5). The reason for this is that, at zero concentration, a channel which is open at time t , and therefore contributing to the macroscopic current, must remain within the set of states \mathcal{E} throughout the interval $[0, t)$. In this situation, the general expression (2.47) can be replaced by

$$I(t) = N\gamma \mathbf{p}_{\mathcal{E}}(0)[\exp(\mathbf{Q}_{\mathcal{E}\mathcal{E}}t)]_{\mathcal{E}\mathcal{A}}\mathbf{u}_{\mathcal{A}}. \tag{2.48}$$

This point is illustrated, and discussed further, in §5.

(h) *Distribution of total open time per burst*

For channels which are *shut* at $t = 0$, the PDF of the total open time within a burst can be found from the obvious modification of Colquhoun & Hawkes (1982, equation (3.22)). Thus,

$$f(t) = \phi_{\mathcal{B}}(0)\mathbf{G}_{\mathcal{B}\mathcal{A}}\exp(\mathbf{V}_{\mathcal{A}\mathcal{A}}t)(-\mathbf{V}_{\mathcal{A}\mathcal{A}})\mathbf{u}_{\mathcal{A}}/P(R \geq 1|\mathcal{F}), \tag{2.49}$$

where

$$\mathbf{V}_{\mathcal{A}\mathcal{A}} = \mathbf{Q}_{\mathcal{A}\mathcal{A}} + \mathbf{Q}_{\mathcal{A}\mathcal{B}}\mathbf{G}_{\mathcal{B}\mathcal{A}}. \tag{2.50}$$

The mean of this distribution is

$$m = \phi_{\mathcal{B}}(0)\mathbf{G}_{\mathcal{B}\mathcal{A}}(-\mathbf{V}_{\mathcal{A}\mathcal{A}})^{-1}\mathbf{u}_{\mathcal{A}}/P(R \geq 1|\mathcal{F}). \tag{2.51}$$

As usual, for channels which are *open* at $t = 0$, similar results can be obtained simply by replacing $\phi_{\mathcal{B}}(0)\mathbf{G}_{\mathcal{B}\mathcal{A}}$ by $\phi_{\mathcal{A}}(0)$ and $P(R \geq 1|\mathcal{F})$ by $P(R \geq 1|\mathcal{A}) = 1$ in (2.49) and (2.51); to get the overall distribution, regardless of initial conditions, replace them instead by $\mathbf{p}_{\mathcal{A}}(0) + \mathbf{p}_{\mathcal{B}}(0)\mathbf{G}_{\mathcal{B}\mathcal{A}}$ and $P(R \geq 1)$.

Clearly, all of these distributions are mixtures of exponentials whose time constants are the reciprocals of the k_A eigenvalues of $-\mathbf{V}_{AA}$.

3. Response of single channel to a pulse of agonist

In this section, we consider the problem of describing how a single channel responds to a pulse of agonist. Specifically, we suppose that a pulse of agonist at a fixed non-zero concentration is applied over the time interval $[0, T)$ so that, within this period, the matrix of transition rates, \mathbf{Q}^1 , is constant. At this concentration, all states communicate so that the matrix is *irreducible*. We assume that the agonist concentration is zero both before and after the pulse, with transition rate matrix \mathbf{Q}^0 . At zero concentration there is an absorbing subset of shut states, \mathcal{C} , as described in § 2.

We consider two possible options about what is recorded: we can start to record the channel behaviour from the end of the pulse, in which case the theory of the preceding section applies with a suitable choice of initial conditions, or we can start recording from the start of the pulse. The advantage of the first approach is that the theory is very much simpler and, if the pulse is short, little information is lost by ignoring what happens within the pulse. If, however, the pulse is long and several openings occur within the pulse then it would be more efficient to start measuring from the start of the pulse. Furthermore, when we come to deal with the problem of time interval omission, in § 4, it turns out that the theory is then more straightforward if we start to record from the start of the pulse.

It is supposed that the pre-pulse period, at zero concentration, is sufficiently long to ensure that the channel has reached the absorbing set, \mathcal{C} , so that the channel is shut (indeed, must be in \mathcal{C}) at $t = 0$ and the occupancies of sets \mathcal{A} and \mathcal{B} are zero, so the partitions of the initial probability vector satisfy

$$\mathbf{p}_A(0) = \mathbf{0}, \quad \mathbf{p}_B(0) = \mathbf{0}, \quad \mathbf{p}_C(0)\mathbf{u}_C = 1.$$

(a) Recording from the end of the pulse

If we start to record from the end of the pulse so that, for example, the latency to the first opening would be the duration of the time interval from T up to the start of the first opening after T , then we can simply apply the theory of § 2, replacing the matrix \mathbf{Q} used throughout that section by \mathbf{Q}^0 . We also need to replace $\mathbf{p}(0)$ of that section by the probability vector

$$\mathbf{p}(T) = \mathbf{p}(0) \exp(\mathbf{Q}^1 T), \quad (3.1)$$

since the set of occupancy probabilities at the end of the pulse becomes the initial probability vector of the jump to zero concentration which occurs at that time. It is useful to think of restarting the clock at the end of the pulse so that this point is treated as time zero as far the future channel behaviour is concerned.

(b) Recording from the start of the pulse

If there is a high probability of openings occurring during the pulse, i.e. the pulse is relatively long compared with average first latency, it should be more informative to record from the start of the pulse. Relatively simple results can be found for the probability distributions of (a) the first latency, (b) the total length of the activation from $t = 0$ to the end of the last opening and (c) the burst length, and these are given here.

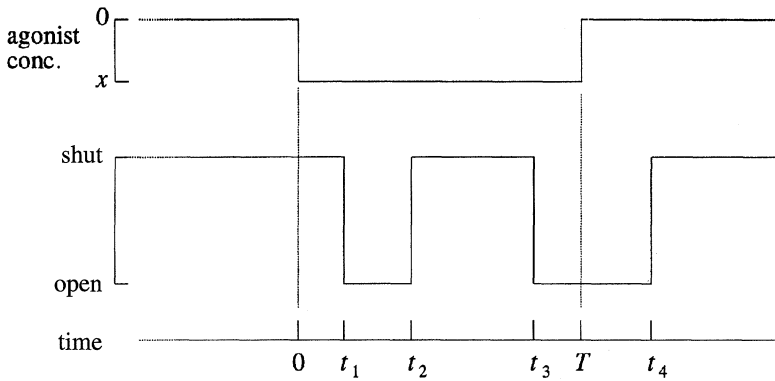


Figure 1. The upper trace shows a pulse of agonist concentration switched on at time 0 and off at time T . The lower trace shows a response in which there are just two openings, one of which is in progress at the end of the pulse, and continues to be open until time t_4 . The channel then closes, never to re-open. The previous times at which opening or closing takes place are denoted t_1 , t_2 and t_3 .

Other distributions (number of openings, length of n th opening, etc.) are more complicated, even without time interval omission, and are discussed in Merlushkin & Hawkes (1995a); these distributions can be calculated numerically. The difficulty comes in taking care of all possible intervals, open or shut, in which the change of agonist concentration at the end of the pulse might fall. Note, however, that it is not difficult to write down the complete likelihood of the process because then we know precisely in which interval the end of pulse falls. The likelihood is very useful for estimating parameters of the model, see, for example, Colquhoun *et al.* (1996). Consider the simple example illustrated in figure 1, in which there are just two openings (beginning at times t_1 , t_3 and ending at times t_2 , t_4) with the end of the pulse taking place at time T , while the second opening is in progress. Then the likelihood of observing this series of events is

$$\begin{aligned}
 & \mathbf{p}_{\mathcal{F}}(0) \mathbf{G}_{\mathcal{F}\mathcal{A}}^1(t_1) \mathbf{G}_{\mathcal{A}\mathcal{F}}^1(t_2 - t_1) \mathbf{G}_{\mathcal{F}\mathcal{A}}^1(t_3 - t_2) \\
 & \times \exp(\mathbf{Q}_{\mathcal{A}\mathcal{A}}^1(T - t_3)) \exp(\mathbf{Q}_{\mathcal{A}\mathcal{A}}^0(t_4 - T)) (-\mathbf{Q}_{\mathcal{A}\mathcal{A}}^0) \mathbf{e}_{\mathbf{b}}^0.
 \end{aligned}$$

In this result, the superscript (0 or 1) indicates which \mathbf{Q} matrix was used in the relevant expression.

(i) *The first latency distribution*

This is an easy generalization of the single jump case. As the channel is assumed shut at $t = 0$, the PDF of the time to first opening (conditional on there being at least one opening) can be written as

$$f_{\text{FL}}(t) = \mathbf{p}_{\mathcal{F}}(0) \mathbf{\Phi}_{\mathcal{F}\mathcal{A}}(t) \mathbf{u}_{\mathcal{A}} / P(R \geq 1), \tag{3.2}$$

where $\mathbf{p}_{\mathcal{F}}(0)$ is the initial distribution over the shut states and $\mathbf{\Phi}_{\mathcal{F}\mathcal{A}}(t)$ is a matrix function, such that its ij th element gives the density (conditional on starting in state i at time 0) of staying in the subset of shut states up to time t and making a transition to open state j at that time. Because of the changing value of the transition rates, we have different expressions for $\mathbf{\Phi}_{\mathcal{F}\mathcal{A}}(t)$, depending on whether the first opening

occurs during or after the end of the pulse. Thus,

$$\left. \begin{aligned} \text{or} \quad \Phi_{\mathcal{F}\mathcal{A}}(t) &= \mathbf{G}_{\mathcal{F}\mathcal{A}}^1(t), \quad 0 \leq t < T \\ \Phi_{\mathcal{F}\mathcal{A}}(t) &= \exp(\mathbf{Q}_{\mathcal{F}\mathcal{F}}^1 T) \mathbf{G}_{\mathcal{F}\mathcal{A}}^0(t - T), \quad t \geq T \end{aligned} \right\} \quad (3.3)$$

where, as in (1.3),

$$\mathbf{G}_{\mathcal{F}\mathcal{A}}^m(t) = \exp(\mathbf{Q}_{\mathcal{F}\mathcal{F}}^m t) \mathbf{Q}_{\mathcal{F}\mathcal{A}}^m, \quad m = 0, 1. \quad (3.4)$$

The second expression in (3.3) represents the matrix product of the probability of survival in the set of shut states, \mathcal{F} , throughout $[0, T]$ and a similar probability of survival in \mathcal{F} for a further period of duration $t - T$, followed by transition to an open state.

Note that $\mathbf{Q}_{\mathcal{F}\mathcal{F}}^0$ is singular, but $\exp(\mathbf{Q}_{\mathcal{F}\mathcal{F}}^0 t)$ can still be evaluated and the first latency PDF obtained. However, it is simpler to use the result in (3.5), which follows from the fact that, at zero concentration, \mathcal{C} is an absorbing subset, so $\mathbf{Q}_{\mathcal{C}\mathcal{A}}^0 = \mathbf{0}$ and $\mathbf{Q}_{\mathcal{C}\mathcal{B}}^0 = \mathbf{0}$. Therefore, we can write

$$\mathbf{G}_{\mathcal{F}\mathcal{A}}^0(t) = \exp(\mathbf{Q}_{\mathcal{F}\mathcal{F}}^0 t) \mathbf{Q}_{\mathcal{F}\mathcal{A}}^0 = \begin{pmatrix} \exp(\mathbf{Q}_{\mathcal{B}\mathcal{B}}^0 t) \mathbf{Q}_{\mathcal{B}\mathcal{A}}^0 \\ \mathbf{0}_{\mathcal{C}\mathcal{A}} \end{pmatrix}. \quad (3.5)$$

Thus we have, for first openings that begin within the pulse,

$$f_{\text{FL}}(t) = \mathbf{p}_{\mathcal{F}}(0) \mathbf{G}_{\mathcal{F}\mathcal{A}}^1(t) \mathbf{u}_{\mathcal{A}} / P(R \geq 1), \quad 0 \leq t < T. \quad (3.6)$$

For $t \geq T$, we can give an alternative expression which makes use of the fact that the channel must exist in the set of \mathcal{B} states at time T if it is ever to open subsequently. Thus,

$$\begin{aligned} f_{\text{FL}}(t) &= \mathbf{p}_{\mathcal{F}}(0) [\exp(\mathbf{Q}_{\mathcal{F}\mathcal{F}}^1 T)]_{\mathcal{F}\mathcal{B}} \exp(\mathbf{Q}_{\mathcal{B}\mathcal{B}}^0(t - T)) \mathbf{Q}_{\mathcal{B}\mathcal{A}}^0 \mathbf{u}_{\mathcal{A}} / P(R \geq 1), \\ &= \{ \mathbf{p}_{\mathcal{B}}(0) [\exp(\mathbf{Q}_{\mathcal{F}\mathcal{F}}^1 T)]_{\mathcal{B}\mathcal{B}} + \mathbf{p}_{\mathcal{C}}(0) [\exp(\mathbf{Q}_{\mathcal{F}\mathcal{F}}^1 T)]_{\mathcal{C}\mathcal{B}} \} \\ &\quad \times \exp(\mathbf{Q}_{\mathcal{B}\mathcal{B}}^0(t - T)) \mathbf{Q}_{\mathcal{B}\mathcal{A}}^0 \mathbf{u}_{\mathcal{A}} / P(R \geq 1), \quad t \geq T \end{aligned} \quad (3.7)$$

or, when the system is entirely in \mathcal{C} at $t = 0$, so $\mathbf{p}_{\mathcal{B}}(0) = \mathbf{0}$, as will usually be the case, this becomes

$$f_{\text{FL}}(t) = \mathbf{p}_{\mathcal{C}}(0) [\exp(\mathbf{Q}_{\mathcal{F}\mathcal{F}}^1 T)]_{\mathcal{C}\mathcal{B}} \exp(\mathbf{Q}_{\mathcal{B}\mathcal{B}}^0(t - T)) \mathbf{Q}_{\mathcal{B}\mathcal{A}}^0 \mathbf{u}_{\mathcal{A}} / P(R \geq 1), \quad t \geq T. \quad (3.8)$$

This shows explicitly that, for $t \geq T$, the time constants are the reciprocals of the $k_{\mathcal{B}}$ eigenvalues of $-\mathbf{Q}_{\mathcal{B}\mathcal{B}}^0$, as in §2. In this case, because $\mathbf{Q}_{\mathcal{C}\mathcal{B}}^0 = \mathbf{0}$, the $\mathbf{Q}_{\mathcal{F}\mathcal{F}}^0$ matrix will be ‘block upper triangular’ and $k_{\mathcal{B}}$ of the $k_{\mathcal{F}}$ eigenvalues of $\mathbf{Q}_{\mathcal{F}\mathcal{F}}^0$ will be identical to the $k_{\mathcal{B}}$ eigenvalues of $\mathbf{Q}_{\mathcal{B}\mathcal{B}}^0$. The remaining $k_{\mathcal{C}}$ eigenvalues of $\mathbf{Q}_{\mathcal{F}\mathcal{F}}^0$ will be the same as those of $\mathbf{Q}_{\mathcal{C}\mathcal{C}}^0$, but these will have zero area in the first latency PDF if the PDF is calculated directly from $\exp(\mathbf{Q}_{\mathcal{F}\mathcal{F}}^0 t)$, as in (3.3). For $t < T$, however, the distribution, using (3.6), can be expressed as a mixture of $k_{\mathcal{F}}$ exponentials whose time constants are the reciprocals of the eigenvalues of $-\mathbf{Q}_{\mathcal{F}\mathcal{F}}^1$.

(ii) *The probability of observing at least one opening*

The probability of observing at least one opening is given by

$$P(R \geq 1) = \int_0^{\infty} \mathbf{p}_{\mathcal{F}}(0) \Phi_{\mathcal{F}\mathcal{A}}(t) \mathbf{u}_{\mathcal{A}} dt. \quad (3.9)$$

Because of the form of $\Phi_{\mathcal{F}\mathcal{A}}(t)$, defined in (3.3), we naturally do the integral in two parts, from 0 to T and from T to ∞ . For the second part of this we must take care because, when the concentration is zero, $\mathbf{Q}_{\mathcal{F}\mathcal{F}}^0$ is singular and it is better to make use of the fact that, in these circumstances, once the set of states \mathcal{C} is reached, the channel can never open again. Thus, $\mathbf{G}_{\mathcal{F}\mathcal{A}}^0(t - T)$ can be calculated as in (3.5), so that we obtain

$$P(R \geq 1) = \mathbf{p}_{\mathcal{F}}(0) \{ (-\mathbf{Q}_{\mathcal{F}\mathcal{F}}^1)^{-1} [\mathbf{I} - \exp(\mathbf{Q}_{\mathcal{F}\mathcal{F}}^1 T)] \mathbf{Q}_{\mathcal{F}\mathcal{A}}^1 + [\exp(\mathbf{Q}_{\mathcal{F}\mathcal{F}}^1 T)]_{\mathcal{F}\mathcal{B}} \mathbf{G}_{\mathcal{B}\mathcal{A}}^0 \} \mathbf{u}_{\mathcal{A}}, \tag{3.10}$$

where, as Colquhoun & Hawkes (1982), we define $\mathbf{G}_{\mathcal{B}\mathcal{A}}^0$ (with the argument t omitted) as

$$\mathbf{G}_{\mathcal{B}\mathcal{A}}^0 = \int_0^\infty \mathbf{G}_{\mathcal{B}\mathcal{A}}^0(t) dt = \int_0^\infty \exp(\mathbf{Q}_{\mathcal{B}\mathcal{B}}^0 t) \mathbf{Q}_{\mathcal{B}\mathcal{A}}^0 dt = (-\mathbf{Q}_{\mathcal{B}\mathcal{B}}^0)^{-1} \mathbf{Q}_{\mathcal{B}\mathcal{A}}^0. \tag{3.11}$$

(iii) *The special case of a simple step to zero concentration*

Suppose now that before time 0 there was a non-zero agonist concentration and a jump to zero concentration occurs at time $t = 0$. In this case, assuming the channel is shut at $t = 0$, so $\mathbf{p}_{\mathcal{F}}(0) \mathbf{u}_{\mathcal{F}} = 1$,

$$f_{\text{FL}}(t) = \mathbf{p}_{\mathcal{F}}(0) \mathbf{G}_{\mathcal{F}\mathcal{A}}^0(t) \mathbf{u}_{\mathcal{A}} / P(R \geq 1 | \mathcal{F}), \tag{3.12}$$

which, from (3.5), can be written as

$$f_{\text{FL}}(t) = \mathbf{p}_{\mathcal{B}}(0) \mathbf{G}_{\mathcal{B}\mathcal{A}}^0(t) \mathbf{u}_{\mathcal{A}} / P(R \geq 1 | \mathcal{F}) = \mathbf{p}_{\mathcal{B}}(0) \exp(\mathbf{Q}_{\mathcal{B}\mathcal{B}}^0 t) \mathbf{Q}_{\mathcal{B}\mathcal{A}}^0(t) \mathbf{u}_{\mathcal{A}} / P(R \geq 1 | \mathcal{F}), \tag{3.13}$$

in agreement with equation (2.39).

(iv) *The special case of a simple step from zero concentration*

For the case of a simple step from zero concentration to a non-zero concentration, $P(R \geq 1)$, calculated from (3.10) with $T \rightarrow \infty$, reduces to

$$P(R \geq 1) = \mathbf{p}_{\mathcal{F}}(0) \mathbf{G}_{\mathcal{F}\mathcal{A}}^1 \mathbf{u}_{\mathcal{A}} = \mathbf{p}_{\mathcal{F}}(0) \mathbf{u}_{\mathcal{F}} = 1,$$

so the PDF in (3.2) reduces to the form given in Colquhoun & Hawkes (1987, equation (6.11)), namely

$$f_{\text{FL}}(t) = \mathbf{p}_{\mathcal{F}}(0) \exp(\mathbf{Q}_{\mathcal{F}\mathcal{F}}^1 t) (-\mathbf{Q}_{\mathcal{F}\mathcal{F}}^1) \mathbf{u}_{\mathcal{F}} / \mathbf{p}_{\mathcal{F}}(0) \mathbf{u}_{\mathcal{F}},$$

except that in this case, $\mathbf{p}_{\mathcal{F}}(0) \mathbf{u}_{\mathcal{F}}$ happens to be equal to 1.

(v) *The distribution of the total activation time*

Total activation time is the time from the start of the pulse to the end of the last opening before the channel is absorbed into the set \mathcal{C} ; again, this will, of course, be conditional on there being at least one opening. It is slightly more complicated than the first latency distribution but superficially similar. The PDF of the length of activation, $f_{\text{LA}}(t)$, can be written as

$$f_{\text{LA}}(t) = \mathbf{p}_{\mathcal{F}}(0) \Psi_{\mathcal{F}\mathcal{F}}(t) \mathbf{c}_{\mathcal{F}}^0 / P(R \geq 1), \tag{3.14}$$

where $\Psi_{\mathcal{F}\mathcal{F}}(t)$ is defined in (3.16)–(3.18) below and $\mathbf{c}_{\mathcal{F}}^0$ is a column vector whose elements give, for each initial shut state, the probability that the system (under the

zero agonist regime) is absorbed into the set \mathcal{C} without any further openings. This is clearly given by

$$c_{\mathcal{C}\mathcal{F}}^0 = \begin{pmatrix} G_{BC}^0 u_c \\ u_c \end{pmatrix}, \tag{3.15}$$

where, similarly to G_{BA}^0 above, $G_{BC}^0 = (-Q_{BB}^0)^{-1}Q_{BC}^0$.

The slightly complicated bit in (3.14) is the definition of $\Psi_{\mathcal{F}\mathcal{F}}(t)$. For $t < T$, its ij th element gives the density (conditional on starting in shut state i at time 0) of a transition from open to shut at time t , after which the channel remains in the set of shut states up to time T , at which point it is in shut state j . Therefore,

$$\Psi_{\mathcal{F}\mathcal{F}}(t) = [\exp(Q^1 t)]_{\mathcal{F}\mathcal{A}} Q_{\mathcal{A}\mathcal{F}}^1 \exp(Q_{\mathcal{F}\mathcal{F}}^1 (T - t)), \quad 0 \leq t < T. \tag{3.16}$$

For $t \geq T$, we need simply the (renewal) density, again conditional on shut state i at time 0, of a transition from the open set to shut state j at time t . Then

$$\Psi_{\mathcal{F}\mathcal{F}}(t) = [\exp(Q^1 T) \exp(Q^0 (t - T))]_{\mathcal{F}\mathcal{A}} Q_{\mathcal{A}\mathcal{F}}^0, \quad t \geq T. \tag{3.17}$$

Note that, for this to happen, the system cannot have been in the set \mathcal{C} at time T otherwise it would never open again (and the activation length would be less than T and thus dealt with by the previous case above); similarly, it must not enter \mathcal{C} throughout the time interval $[T, t)$. Thus, the expression above can be written alternatively as

$$\Psi_{\mathcal{F}\mathcal{F}}(t) = [\exp(Q^1 T)]_{\mathcal{F}\mathcal{E}} [\exp(Q_{\mathcal{E}\mathcal{E}}^0 (t - T))]_{\mathcal{E}\mathcal{A}} Q_{\mathcal{A}\mathcal{F}}^0, \quad t \geq T, \tag{3.18}$$

where the subset \mathcal{E} is defined as $\mathcal{E} = \mathcal{A} \cup \mathcal{B}$.

(vi) *The distribution of the burst length*

The burst is defined as the interval from the start of the first opening to the end of the last opening, so that the burst length is such that

$$\text{length of activation} = \text{first latency} + \text{burst length}.$$

First, we shall find an expression for the joint probability density of the first latency and the burst length conditional, as usual, on there being at least one opening. This can be expressed somewhat like equation (3.14) as

$$f_{\text{FB}}(t_1, t) = p_{\mathcal{F}}(0) \tilde{\Xi}_{\mathcal{F}\mathcal{F}}(t_1, t) c_{\mathcal{F}}^0 / P(R \geq 1), \tag{3.19}$$

where t_1 refers to first latency and t to burst length. As with the matrix $\Psi_{\mathcal{F}\mathcal{F}}(t)$, the definition of the matrix density $\tilde{\Xi}_{\mathcal{F}\mathcal{F}}(t_1, t)$ takes different forms, depending on whether things happen before or after the end of the pulse.

(1) When $t_1 + t < T$, so the total activation is completed within the pulse, the ij th element represents a bivariate probability density of the event that, conditional on the channel being in shut state i at time 0, the first transition to the open subset occurs at time t_1 , there is a transition from open to shut states at time $t_1 + t$ and the channel remains within the set of shut states from then to time T , at which point it is in shut state j . Then

$$\tilde{\Xi}_{\mathcal{F}\mathcal{F}}(t_1, t) = \Phi_{\mathcal{F}\mathcal{A}}(t_1) [\exp(Q^1 t)]_{\mathcal{A}\mathcal{A}} Q_{\mathcal{A}\mathcal{F}}^1 \exp(Q_{\mathcal{F}\mathcal{F}}^1 (T - t - t_1)). \tag{3.20}$$

(2) When $t_1 + t \geq T$, so that activation finishes after the end of the pulse, the ij th element of the matrix represents a bivariate probability density of the event that,

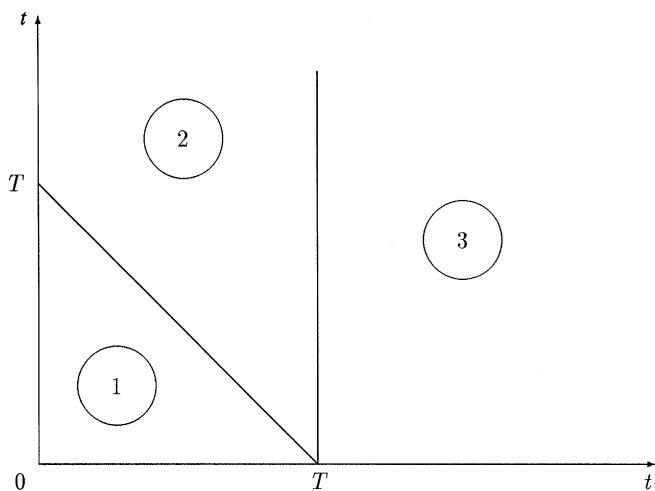


Figure 2. The figure illustrates the domains in which the three analytical forms of the joint matrix density $\tilde{\Xi}$ obtain, leading to the three matrix integrals I_k whose corresponding subscripts ($k = 1, 2, 3$) are marked on the figure.

conditional on the channel being in shut state i at time 0, the first transition to the open subset occurs at time t_1 and there is a transition from open to shut states at time $t_1 + t$, with the channel entering shut state j .

Then for $t_1 < T \leq t_1 + t$, so that the burst straddles the end of the pulse, and noting that the channel must not enter the set \mathcal{C} during the time interval between the end of the pulse and the end of the burst, we obtain

$$\tilde{\Xi}_{\mathcal{F}\mathcal{F}}(t_1, t) = \Phi_{\mathcal{F}\mathcal{A}}(t_1)[\exp(\mathbf{Q}^1(T - t_1))]_{\mathcal{A}\mathcal{E}}[\exp(\mathbf{Q}_{\mathcal{E}\mathcal{E}}^0(t - T + t_1))]_{\mathcal{E}\mathcal{A}}\mathbf{Q}_{\mathcal{A}\mathcal{F}}^0. \quad (3.21)$$

(3) When $t_1 \geq T$, so the first opening occurs after the end of the pulse, the definition is the same as for case 2 above, but we have the simpler expression

$$\tilde{\Xi}_{\mathcal{F}\mathcal{F}}(t_1, t) = \Phi_{\mathcal{F}\mathcal{A}}(t_1)[\exp(\mathbf{Q}_{\mathcal{E}\mathcal{E}}^0(t))]_{\mathcal{A}\mathcal{A}}\mathbf{Q}_{\mathcal{A}\mathcal{F}}^0. \quad (3.22)$$

Thus there are three regions, represented in figure 2, in which the matrix function takes different forms.

To get the marginal density of burst length we must integrate the joint density (3.19) with respect to t_1 , giving

$$f_{\text{BL}}(t) = \mathbf{p}_{\mathcal{F}}(0)\Xi_{\mathcal{F}\mathcal{F}}(t)^{\mathcal{C}}\mathbf{c}_{\mathcal{F}}^0/P(R \geq 1), \quad (3.23)$$

where

$$\Xi_{\mathcal{F}\mathcal{F}}(t) = \int_0^\infty \tilde{\Xi}_{\mathcal{F}\mathcal{F}}(t_1, t) dt_1. \quad (3.24)$$

Since the bivariate density has three different analytical forms, this integral can be written as

$$\begin{aligned} \Xi_{\mathcal{F}\mathcal{F}}(t) &= \mathbf{I}_1(t) + \mathbf{I}_2(t) + \mathbf{I}_3(t), \quad \text{for } 0 \leq t < T, \\ \Xi_{\mathcal{F}\mathcal{F}}(t) &= \mathbf{I}_2(t) + \mathbf{I}_3(t), \quad \text{for } t \geq T. \end{aligned} \quad (3.25)$$

The \mathbf{I} functions are integrals over the respective domains where, as can be seen

from figure 2,

$$\left. \begin{aligned} I_1(t) &= \int_0^{T-t} \tilde{\Xi}_{\mathcal{F}\mathcal{F}}(t_1, t) dt_1, \quad t < T, \\ I_2(t) &= \int_{\max(0, T-t)}^T \tilde{\Xi}_{\mathcal{F}\mathcal{F}}(t_1, t) dt_1, \quad \text{for all } t \geq 0, \\ I_3(t) &= \int_T^\infty \tilde{\Xi}_{\mathcal{F}\mathcal{F}}(t_1, t) dt_1, \quad \text{for all } t \geq 0. \end{aligned} \right\} \quad (3.26)$$

The functional forms for the three integrands above are given in (3.20)–(3.22). Explicit results for these are somewhat tedious and are given in Merlushkin & Hawkes (1995*a*). We merely note here that, for $t \geq T$, the resulting density is a mixture of $k_{\mathcal{E}}$ exponential terms whose time constants are the reciprocals of the eigenvalues of $-Q_{\mathcal{E}\mathcal{E}}^0$. Programs for evaluating them are available.

We note that this approach yields an alternative derivation of the total activation time, because $\Psi_{\mathcal{F}\mathcal{F}}(t)$, which was given in (3.16)–(3.18), clearly satisfies

$$\Psi_{\mathcal{F}\mathcal{F}}(t) = \int_0^t \tilde{\Xi}_{\mathcal{F}\mathcal{F}}(t_1, t - t_1) dt_1. \quad (3.27)$$

4. Allowing for time interval omission

It is well known that in experiments, due to limitations of the equipment, we cannot detect short openings and shuttings of a channel; the so-called time interval omission problem. This is taken into account by defining an ‘apparent opening’ as a period which begins with a sojourn in the set of open states, \mathcal{A} , in excess of some dead time, ξ , and possibly followed by an alternating series of shuttings and openings in which the shut times are all less than ξ . The apparent open time ends at the start of a period in the shut states, \mathcal{F} , in excess of ξ which forms the beginning of a similarly defined ‘apparent shut time’. The treatment of this problem has been considered by several authors including Ball & Sansom (1988), Hawkes *et al.* (1990, 1992) and Jalali & Hawkes (1992). The apparent distributions are often considerably different from those predicted by the classical theory which does not take these omissions into account.

(a) *Recording from the start of the pulse*

In this section, we give expressions for the distributions of apparent first latency in a form similar to that for the classical case treated in the previous section. Of course, the meaning of constituent parts of the expressions and their computation become more complicated. The distribution of the apparent length of activation is somewhat more complicated still. Therefore, we do not present explicit results here and refer interested readers to Merlushkin & Hawkes (1995*a*) for details.

We assume that the channel remains entirely in the set of shut states before the beginning of the pulse and, therefore, an apparent shut sojourn is in progress at time 0, the channel being in one of the shut states. The probabilities of being in each of the shut states are given by the elements of the vector $\mathbf{p}_{\mathcal{F}}(0)$.

(i) *The distribution of the apparent first latency*

The apparent first latency is defined as the time interval from the beginning of the pulse up to the beginning of the first apparent opening of the channel. We consider a

PDF conditional on there being at least one apparent opening. In a manner similar to equation (3.2), we can write

$$f_{\text{AFL}}(t) = \mathbf{p}_{\mathcal{F}}(0)\hat{\Phi}_{\mathcal{F}\mathcal{A}}(t)\mathbf{u}_{\mathcal{A}}/P(\hat{R} \geq 1), \tag{4.1}$$

where \hat{R} is a random variable denoting the number of apparent openings before absorption into the set of states \mathcal{C} and $\hat{\Phi}_{\mathcal{F}\mathcal{A}}(t)$ is a matrix such that its ij th element gives the density (conditional on starting in shut state i at time 0) of having no ‘long’ sojourns (of length $\geq \xi$) in open states throughout the time interval $[0, t)$, making transition from shut to open states at time t , and staying in the set of open states for at least ξ following this transition so that, at time $t + \xi$, the channel is in open state j .

Due to the step-wise nature of the underlying process, we have different expressions for $\hat{\Phi}_{\mathcal{F}\mathcal{A}}(t)$ for different time intervals. First,

$$\hat{\Phi}_{\mathcal{F}\mathcal{A}}(t) = {}^{\mathcal{F}}\mathbf{R}^1(t)\mathbf{Q}_{\mathcal{F}\mathcal{A}}^1 \exp(\mathbf{Q}_{\mathcal{A}\mathcal{A}}^1\xi), \quad 0 \leq t < T - \xi, \tag{4.2}$$

where we denote by ${}^{\mathcal{F}}\mathbf{R}^1(t)$ the matrix introduced in (1.13), whose computation is discussed in the Appendix, derived from the generator matrix \mathbf{Q}^1 instead of \mathbf{Q} . Note that, as implied in the above equation, we assume that the pulse length, T , is greater than the dead time, ξ .

For $T - \xi \leq t < T$, the expression is slightly more complicated since the open sojourn which follows the first apparent shut time has to be split into two subintervals, before and after the end of pulse, where the generator matrix of the underlying process is equal to \mathbf{Q}^1 and \mathbf{Q}^0 , respectively. Then

$$\hat{\Phi}_{\mathcal{F}\mathcal{A}}(t) = {}^{\mathcal{F}}\mathbf{R}^1(t)\mathbf{Q}_{\mathcal{F}\mathcal{A}}^1 \exp(\mathbf{Q}_{\mathcal{A}\mathcal{A}}^1(T-t)) \exp(\mathbf{Q}_{\mathcal{A}\mathcal{A}}^0(t+\xi-T)), \quad T - \xi \leq t < T. \tag{4.3}$$

For $t \geq T$, the interval where the process has ‘no resolvable open sojourns’ starts at time 0 and terminates after the end of pulse. We now have to split this interval in a manner similar to that above to operate on homogeneous subintervals. At the change point T , we can either be in a shut state or within a ‘short’ open sojourn which started at time $(T - r_0)$, $0 \leq r_0 < \xi$, and finishes at time $T + r_1$, $0 \leq r_1 < \xi - r_0$. Adding up all these possibilities, we obtain an expression for the density of interest

$$\begin{aligned} \hat{\Phi}_{\mathcal{F}\mathcal{A}}(t) = & \left\{ {}^{\mathcal{F}}\mathbf{R}^1(T){}^{\mathcal{F}}\mathbf{R}^0(t-T) + \int_0^\xi dr_0 {}^{\mathcal{F}}\mathbf{R}^1(T-r_0)\mathbf{Q}_{\mathcal{F}\mathcal{A}}^1 \exp(\mathbf{Q}_{\mathcal{A}\mathcal{A}}^1r_0) \right. \\ & \times \left. \int_0^{\min(\xi-r_0, t-T)} dr_1 \exp(\mathbf{Q}_{\mathcal{A}\mathcal{A}}^0r_1)\mathbf{Q}_{\mathcal{A}\mathcal{F}}^0{}^{\mathcal{F}}\mathbf{R}^0(t-(T+r_1)) \right\} \\ & \times \mathbf{Q}_{\mathcal{F}\mathcal{A}}^0 \exp(\mathbf{Q}_{\mathcal{A}\mathcal{A}}^0\xi), \quad \text{for } t \geq T. \end{aligned} \tag{4.4}$$

The matrix function ${}^{\mathcal{F}}\mathbf{R}^0(t)$ is similar to ${}^{\mathcal{F}}\mathbf{R}^1(t)$, but is based on the generator matrix \mathbf{Q}^0 . Although the appearance of this last part of $\hat{\Phi}_{\mathcal{F}\mathcal{A}}(t)$ is messy, it involves only integrations over intervals of length $< \xi$ and its evaluation by numerical integration is simple. For a short interval with an integrand which does not change much, we found the simple Trapezium Rule quite adequate.

(ii) *The probability of observing at least one apparent opening*

The probability of observing at least one apparent opening can be obtained in the same way as for the classical case, i.e. by integrating the numerator of $f_{\text{AFL}}(t)$ in (4.1) over t . Here we approach it differently and calculate $P(\hat{R} = 0) = 1 - P(\hat{R} \geq 1)$ instead.

Let us introduce a column vector of probabilities, similar to (3.15). Elements of this vector give, for each initial shut state, the probability that the system (under the zero agonist regime) is absorbed into the set \mathcal{C} without any prior 'long' openings. This is clearly given by

$$c_{\mathcal{F}}^0 = \begin{pmatrix} {}^{\mathcal{B}}\mathbf{R}^0 \mathbf{Q}_{\mathcal{B}\mathcal{C}}^0 u_{\mathcal{C}} \\ u_{\mathcal{C}} \end{pmatrix}, \quad (4.5)$$

where, by analogy with the standard ${}^{\mathcal{F}}\mathbf{R}^0(t)$, matrix function ${}^{\mathcal{B}}\mathbf{R}^0(t)$ gives 'survival' probabilities of having no 'long' sojourns outside \mathcal{B} (i.e. in $\mathcal{A} \cup \mathcal{C}$) during $[0, t)$ and

$${}^{\mathcal{B}}\mathbf{R}^0 = \int_0^{\infty} dt {}^{\mathcal{B}}\mathbf{R}^0(t). \quad (4.6)$$

Now, making use of this new probability vector, the probability of having no apparent openings can be calculated as

$$P(\hat{R} = 0) = p_{\mathcal{F}}(0) \left\{ {}^{\mathcal{F}}\mathbf{R}^1(T) + \int_0^{\xi} dr_0 {}^{\mathcal{F}}\mathbf{R}^1(T - r_0) \mathbf{Q}_{\mathcal{F}\mathcal{A}}^1 \exp(\mathbf{Q}_{\mathcal{A}\mathcal{A}}^1 r_0) \right. \\ \left. \times \int_0^{\xi - r_0} dr_1 \exp(\mathbf{Q}_{\mathcal{A}\mathcal{A}}^0 r_1) \mathbf{Q}_{\mathcal{A}\mathcal{F}}^0 \right\} c_{\mathcal{F}}^0. \quad (4.7)$$

This expression simply adds up probabilities of having no apparent openings and, at time T , the underlying process either being in the shut subset or being within a 'short' opening which starts at $(T - r_0)$ and finishes at $(T + r_1)$.

(iii) *The distribution of the apparent length of an activation*

Apparent length of activation is defined as a time interval from the beginning of the pulse up to the end of the last apparent opening before absorption. Again, in a superficially simple way, we can write

$$f_{\text{ALA}}(t) = p_{\mathcal{F}}(0) \hat{\Psi}_{\mathcal{F}\mathcal{F}}(t) c_{\mathcal{F}}^0 / P(R \geq 1). \quad (4.8)$$

The definition of the matrix function $\hat{\Psi}_{\mathcal{F}\mathcal{F}}(t)$ is similar to the definition of $\Psi_{\mathcal{F}\mathcal{F}}(t)$ for the classical case, with 'apparent' events replacing the corresponding classical transitions and sojourns. Explicit formulae for this density can be obtained using similar arguments to those for $\hat{\Phi}_{\mathcal{F}\mathcal{A}}(t)$. However, they are cumbersome and are not included in this paper but can be found in Merlushkin & Hawkes (1995a).

(iv) *Distributions of other characteristics*

Other features that we have considered earlier, such as the distributions of burst length, number of openings and the length of the r th opening are much more complicated in the case of time interval omission and have not been attempted. Note, however, that it is still possible to compute the complete likelihood of the process in a manner similar to the method described in Colquhoun *et al.* (1996), which generalizes the example without time interval omission discussed in relation to figure 1. This uses semi-Markov kernels, essentially rather more complicated versions of $\mathbf{G}_{\mathcal{A}\mathcal{F}}(t)$ and $\mathbf{G}_{\mathcal{F}\mathcal{A}}(t)$ defined in (1.3), which are discussed in Merlushkin & Hawkes (1995a, 1997).

(b) *Recording from the end of the pulse*

It might be thought that, as in §3, it would be easier to deal with the theory to describe what happens if you start recording from the end of the pulse instead of the

beginning. Indeed, all the problems that make the earlier part of this section hard disappear, because the agonist concentration would be constant over the observation period. However, we get another problem instead. The problem now is that, when dealing with apparent times, we need to know at the time the concentration changes whether an open period, or a shut period, *appears* to be in progress, and for how long; the sort of thing that makes equation (4.4) complicated. The advantage of starting at the beginning of the pulse is that we *know* the channel is shut, both *really* and *apparently*, at $t = 0$.

Progress on this problem, and the related one of a single jump in concentration, needs some consideration about how the signal is to be processed in the neighbourhood of the jump, both before and after.

(c) *Response to a single jump*

We now consider briefly the effect of time interval omission on the case of a single jump, mentioning two special cases.

(i) *The special case of a single jump from zero concentration*

In this case, we know that the channel must be in the set of shut states at $t = 0$ and has been there for some time. With non-zero agonist concentration after the jump, at least one apparent open time is certain to occur (indeed there will be infinitely many, so that the burst length and total activation will be infinite). Then the latency to the first opening has PDF given by (4.1), with $P(\hat{R} \geq 1) = 1$, and with $\hat{\Phi}_{\mathcal{F}\mathcal{A}}(t)$ given by (4.2) for all $t \geq 0$. In other words,

$$f_{\text{AFL}}(t) = \mathbf{p}_{\mathcal{F}}(0) \mathcal{F} \mathbf{R}^1(t) \mathbf{Q}_{\mathcal{F}\mathcal{A}}^1 \exp(\mathbf{Q}_{\mathcal{A}\mathcal{A}}^1 \xi) \mathbf{u}_{\mathcal{A}}, \quad t \geq 0. \tag{4.9}$$

Following Hawkes *et al.* (1990, equation (3.2)), we can replace the matrix function $\mathbf{G}_{\mathcal{A}\mathcal{F}}(t)$ defined in (1.3) by

$${}^e \mathbf{G}_{\mathcal{A}\mathcal{F}}^1(t) = {}^A \mathbf{R}^1(t - \xi) \mathbf{Q}_{\mathcal{A}\mathcal{F}}^1 \exp(\mathbf{Q}_{\mathcal{F}\mathcal{F}}^1 \xi), \quad t \geq \xi, \tag{4.10}$$

and let $\mathbf{G}_{\mathcal{A}\mathcal{F}}$ be replaced by

$${}^e \mathbf{G}_{\mathcal{A}\mathcal{F}}^1 = \int_0^\infty {}^e \mathbf{G}_{\mathcal{A}\mathcal{F}}^1(t) dt = \{ \mathbf{I} - \mathbf{G}_{\mathcal{A}\mathcal{F}}^1 (\mathbf{I} - \exp(\mathbf{Q}_{\mathcal{F}\mathcal{F}}^1 \xi)) \mathbf{G}_{\mathcal{F}\mathcal{A}}^1 \}^{-1} \mathbf{G}_{\mathcal{A}\mathcal{F}}^1 \exp(\mathbf{Q}_{\mathcal{F}\mathcal{F}}^1 \xi). \tag{4.11}$$

Similar results are obtained by interchanging \mathcal{A} and \mathcal{F} .

Then the distribution of the n th apparent shut time has PDF

$$f(t) = \mathbf{p}_{\mathcal{F}}(0) ({}^e \mathbf{G}_{\mathcal{F}\mathcal{A}}^1 {}^e \mathbf{G}_{\mathcal{A}\mathcal{F}}^1)^{n-1} {}^e \mathbf{G}_{\mathcal{F}\mathcal{A}}^1(t) \mathbf{u}_{\mathcal{A}}, \quad t \geq \xi, \quad n \geq 2. \tag{4.12}$$

Similarly, the distribution of the n th apparent open time has PDF

$$f(t) = \mathbf{p}_{\mathcal{F}}(0) ({}^e \mathbf{G}_{\mathcal{F}\mathcal{A}}^1 {}^e \mathbf{G}_{\mathcal{A}\mathcal{F}}^1)^{n-1} {}^e \mathbf{G}_{\mathcal{F}\mathcal{A}}^1 {}^e \mathbf{G}_{\mathcal{A}\mathcal{F}}^1(t) \mathbf{u}_{\mathcal{F}}, \quad t \geq \xi, \quad n \geq 1. \tag{4.13}$$

Notice that, unlike the apparent open times or subsequent apparent shut times, the first apparent shut time (or apparent latency) may take values less than the deadtime, ξ . This is because, with zero concentration before the jump, we are sure that the channel is shut initially and do not require an initial sojourn in \mathcal{F} to be greater than ξ in order to convince us that we have an apparent shut time in progress. Consequently, the density $f_{\text{AFL}}(t)$, see equation (4.9), depends on $\mathcal{F} \mathbf{R}^1(t)$ and its asymptotic form will depend on the asymptotic form of $\mathcal{F} \mathbf{R}^1(t)$ and therefore will normally be used for $t > 2\xi$ (see Appendix). However, all other apparent open time

or shut times must start with a true open (or shut) time greater than ξ and so the probability densities $f(t)$ in (4.12) and (4.13) depend on ${}^{\mathcal{F}}\mathbf{R}^1(t - \xi)$ or ${}^{\mathcal{A}}\mathbf{R}^1(t - \xi)$. These \mathbf{R} matrices have asymptotic approximations which will normally be used for $u = t - \xi > 2\xi$ and hence $f(t)$ has an asymptotic form applied for $t > 3\xi$.

(ii) *Mean apparent sojourns*

Means of apparent shut times or apparent open times may be obtained, for example, by replacing ${}^e\mathbf{G}_{\mathcal{F}\mathcal{A}}^1(t)$ or ${}^e\mathbf{G}_{\mathcal{A}\mathcal{F}}^1(t)$ by appropriate constant matrices, respectively $\mathbf{M}_{\mathcal{F}\mathcal{A}}^1$ and $\mathbf{M}_{\mathcal{A}\mathcal{F}}^1$, say, as in Colquhoun *et al.* (1996). Formulae for these matrices may be calculated as shown in the Appendix, replacing the general matrix \mathbf{Q} by the particular matrix, here denoted \mathbf{Q}^1 .

Ball *et al.* (1989) showed that the n th apparent mean has the form

$$m_n = \sum_{i=1}^{N_g} \rho_i^{n-1} w_i, \quad (4.14)$$

where N_g is the minimum of the number of open gateway states and the number of shut gateway states. The above result holds for both apparent shut and apparent open times with the *same* ρ_i , which are the non-zero eigenvalues of ${}^e\mathbf{G}_{\mathcal{F}\mathcal{A}}^1 {}^e\mathbf{G}_{\mathcal{A}\mathcal{F}}^1$, from using the spectral expansion of the term $({}^e\mathbf{G}_{\mathcal{F}\mathcal{A}}^1 {}^e\mathbf{G}_{\mathcal{A}\mathcal{F}}^1)^{n-1}$ which occurs in both (4.12) and (4.13); the constants w_i will, however, be different. Thus, if the spectral expansion is

$$({}^e\mathbf{G}_{\mathcal{F}\mathcal{A}}^1 {}^e\mathbf{G}_{\mathcal{A}\mathcal{F}}^1)^{n-1} = \sum_{i=1}^{N_g} \rho_i^{n-1} \mathbf{A}_i, \quad (4.15)$$

substitution into (4.12) yields the form (4.14) for the mean of the n th apparent shut time with

$$w_i = \mathbf{p}_{\mathcal{F}}(0) \mathbf{A}_i \mathbf{M}_{\mathcal{F}\mathcal{A}}^1 \mathbf{u}_{\mathcal{A}}, \quad i = 1 \text{ to } N_g; \quad (4.16)$$

substitution into (4.13) yields the same form (4.14) for the mean of the n th apparent open time, but with the weights now given by

$$w_i = \mathbf{p}_{\mathcal{F}}(0) \mathbf{A}_i {}^e\mathbf{G}_{\mathcal{F}\mathcal{A}}^1 \mathbf{M}_{\mathcal{A}\mathcal{F}}^1 \mathbf{u}_{\mathcal{F}}, \quad i = 1 \text{ to } N_g. \quad (4.17)$$

Note that one eigenvalue, say ρ_1 , is always unity and the others have modulus less than 1, so that the equilibrium mean value is given by $m_{\infty} = \lim_{n \rightarrow \infty} m_n = w_1$.

(iii) *Jump from non-zero concentration*

As mentioned above, in connection with recording from the end of a pulse, considerable complications arise when the agonist concentration is non-zero before the jump. You need to keep track of whether the channel is apparently open or apparently shut at the time of the jump, and for how long. Merlushkin & Hawkes (1995b) obtain the distribution of the apparent first latency.

In the case where the agonist concentration is zero after the jump, they also obtain the distribution of total apparent activation. The results are far too complex to present here, but we remark that software to compute these distributions numerically has been developed. In this case also, or any other situation where there is an absorbing set of shut states \mathcal{C} after the jump (for example due to desensitization), distributions of the n th apparent open time and n th apparent shut time, generalising equations (2.24) and (2.32) to the time interval omission situation, can be found by methods used for studying bursts in Hawkes & Merlushkin (1996), or Merlushkin (1996).

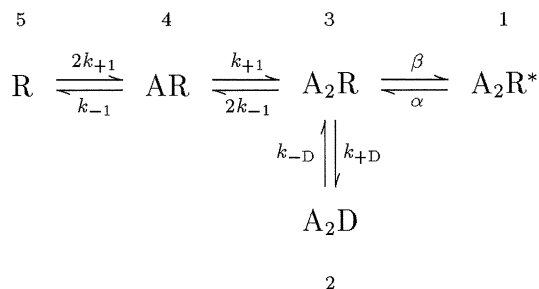


Figure 3. Mechanism used for numerical examples. The open channel corresponds to the single state 1, the other four states correspond to a shut channel. Agonist binding steps are needed to move from state 5 to state 4 and from state 4 to state 3, the reverse transitions being the result of dissociation. All other transitions are the result of conformation changes. The values for the rate constants used for the calculations are $k_{+1} = 5 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$, $k_{-1} = 4.7 \text{ s}^{-1}$, $k_{+D} = 8.4 \text{ s}^{-1}$, $k_{-D} = 1.8 \text{ s}^{-1}$, $\alpha = 916 \text{ s}^{-1}$ and $\beta = 46.5 \text{ s}^{-1}$.

5. A numerical example

To give a simple numerical example, we consider the mechanism discussed in Colquhoun & Hawkes (1995c), which was proposed for the NMDA receptor by Lester & Jahr (1992), and illustrated in figure 3. It has four shut states and only one open state, so there are no correlations between the durations of successive intervals. It is, therefore, too simple to account for most single channel results although it can describe the time course of macroscopic currents quite well. There are two sequential bindings of agonist molecules (*A*) to the shut receptor, to produce A_2R , which may then either open (to A_2R^*), or enter the long-lived shut ('desensitized') state, A_2D . For the purposes of this paper, we have modified the rate, α , at which the open state shuts, by a factor of ten, so that open times are much shorter and the effect of time interval omission can be seen more clearly.

The matrix of transition rates is given algebraically by

$$\mathbf{Q}(x) = \begin{bmatrix} -\alpha & 0 & \alpha & 0 & 0 \\ 0 & -k_{-D} & k_{-D} & 0 & 0 \\ \beta & k_{+D} & -(\beta + k_{+D} + 2k_{-1}) & 2k_{-1} & 0 \\ 0 & 0 & k_{+1}x & -(k_{-1} + k_{+1}x) & k_{-1} \\ 0 & 0 & 0 & 2k_{+1}x & -2k_{+1}x \end{bmatrix}, \quad (5.1)$$

where \mathbf{Q} has been expressed as a function of the agonist concentration, x . If we take $x = 1 \text{ mM}$, and use the rate constants given in the legend of figure 3, we get an irreducible matrix of transition rates

$$\mathbf{Q}^1 = \mathbf{Q}(0.001) = \begin{bmatrix} -0.916 & 0 & 0.916 & 0 & 0 \\ 0 & -0.0018 & 0.0018 & 0 & 0 \\ 0.0465 & 0.0084 & -0.0643 & 0.0094 & 0 \\ 0 & 0 & 5 & -5.0047 & 0.0047 \\ 0 & 0 & 0 & 10 & -10 \end{bmatrix}, \quad (5.2)$$

where the timescale is measured in milliseconds, so \mathbf{Q} has units of ms^{-1} .

At zero concentration, we get a reducible matrix

$$Q^0 = Q(0) = \left[\begin{array}{c|cc|cc} -0.916 & 0 & 0.916 & 0 & 0 \\ \hline 0 & -0.0018 & 0.0018 & 0 & 0 \\ 0.0465 & 0.0084 & -0.0643 & 0.0094 & 0 \\ \hline 0 & 0 & 0 & -0.0047 & 0.0047 \\ 0 & 0 & 0 & 0 & 0 \end{array} \right], \quad (5.3)$$

where we have also shown the partition into the three sets of states. There is one open state, $\mathcal{A} = \{1\}$; at zero concentration, $\mathcal{C} = \{4, 5\}$ is an absorbing set of states, R and AR , because at least one binding step is needed to reach state 3 (A_2R) which would allow the possibility of opening by a subsequent conformation change. This binding is clearly impossible in the absence of agonist. On the other hand, it is possible to reach the open state from set $\mathcal{B} = \{2, 3\}$, i.e. from A_2R or A_2D , even in the absence of agonist.

(a) *Channel behaviour after a jump to zero concentration*

We will use the above mechanism to illustrate the results of §2. Supposing that we pre-equilibrate with 1 mM agonist, then the matrix Q^1 applies before time zero, so we take the corresponding vector of equilibrium probabilities as the initial vector at the time of the jump;

$$p(0) = [0.00888 \mid 0.81595 \quad 0.17485 \mid 0.00033 \quad 1.55 \times 10^{-7}], \quad (5.4)$$

again shown partitioned into $p_{\mathcal{A}}(0)$, $p_{\mathcal{B}}(0)$ and $p_{\mathcal{C}}(0)$. On average, 81.6% of channels are in the 'desensitized' state. Then the various initial probabilities calculated from these, as given by (2.1)–(2.4) and following (2.16), are

$$P(\mathcal{A}) = 0.00888, \quad P(\mathcal{B}) = 0.99080, \quad P(\mathcal{C}) = 0.00033, \quad P(\mathcal{F}) = 0.99112,$$

with conditional probabilities

$$P(\mathcal{B}|\mathcal{F}) = 0.99967, \quad P(\mathcal{C}|\mathcal{F}) = 0.00033,$$

$$\phi_{\mathcal{A}}(0) = [1], \quad \phi_{\mathcal{B}}(0) = [0.82326 \quad 0.17641].$$

Thus, initially, the channel is highly likely to be shut, but in subset \mathcal{B} (especially state 2, A_2D) rather than \mathcal{C} , and hence potentially able to open *after* the jump to zero agonist concentration.

As in §2, the remaining calculations, relating to behaviour after the jump, are made on the basis of the matrix of transition rates $Q = Q^0$. The transition probability matrices, calculated as in (1.12), are

$$G_{\mathcal{A}\mathcal{B}} = [0 \quad 1], \quad G_{\mathcal{B}\mathcal{A}} = \begin{bmatrix} 0.83184 \\ 0.83184 \end{bmatrix}, \quad G_{\mathcal{B}\mathcal{C}} = \begin{bmatrix} 0.16816 & 0 \\ 0.16816 & 0 \end{bmatrix}. \quad (5.5)$$

$G_{\mathcal{A}\mathcal{B}}$ reflects the fact that shutting is just a transition from state 1 to state 3. Also, the only way to leave set \mathcal{B} is via state 3 (A_2R), moving to state 1 (opening) with probability 0.83184 and dissociating to state 4 (AR) with probability 0.16816, and this is true whether the channel starts in state 2 or in state 3 (so that the rows of

Table 1.

condition	$P(\text{no openings})$	$P(\text{at least one opening})$	expected number of openings
shut initially	$P(R = 0 \mathcal{F}) = 0.16843$	$P(R \geq 1 \mathcal{F}) = 0.83157$	$E(R \mathcal{F}) = 4.945$
open initially	$P(R = 0 \mathcal{A}) = 0$	$P(R \geq 1 \mathcal{A}) = 1$	$E(R \mathcal{A}) = 5.947$
overall	$P(R = 0) = 0.16694$	$P(R \geq 1) = 0.83306$	$E(R) = 4.954$

$\mathbf{G}_{\mathcal{B}\mathcal{A}}$ are identical, as are those of $\mathbf{G}_{\mathcal{B}\mathcal{C}}$. The end-of-burst vector, given by (2.10), is just the singleton

$$\mathbf{e}_b = [0.16816].$$

This contains the same probability as in $\mathbf{G}_{\mathcal{B}\mathcal{C}}$, because the only way to get from \mathcal{A} to \mathcal{C} without re-opening is to make the series of transitions $1 \rightarrow 3 (\dots 3 \rightarrow 2 \rightarrow 3 \dots) 3 \rightarrow 4$, where there may be any number of $3 \rightarrow 2 \rightarrow 3$ oscillations within the set \mathcal{B} between first entering state 3 and finally leaving it for state 4.

(i) *The number of openings*

The expected number of openings, and the probability of seeing any at all, after the jump can be found under various initial conditions from (2.5)–(2.7), (2.12)–(2.15) and (2.20). They are shown in table 1.

So it is quite likely that there will be some openings after the jump; on average, about five or six. Note that the mean number of openings conditional on at least one occurring, μ_1 from (2.15), is 5.947, the same as $E(R|\mathcal{A})$, because a consequence of having only one open state is that, as soon as the first opening occurs, the future behaviour is independent of the situation at time zero.

Because there is only one open state, the probability distribution of the number of openings, from (2.9), (2.16) or (2.18), is a modified geometric distribution of the form

$$p(r) = \begin{cases} (1 - \alpha)q^{r-1}p, & r \geq 1, \\ \alpha, & r = 0, \end{cases} \tag{5.6}$$

where $q = 0.83184$, from $\mathbf{G}_{\mathcal{B}\mathcal{A}}$ in (5.5), is the probability that a channel in the set \mathcal{B} will reopen, and $p = 1 - q$. The value of α is $P(R = 0|\mathcal{F})$, $P(R = 0|\mathcal{A})$ or $P(R = 0)$, from table 1, depending on whether we condition on the channel being shut or open initially, or treat it unconditionally. Some numerical probabilities are given in table 2.

Thus, although the average is five or six openings, the distribution has quite a long tail so that, for example, 15–20% of jumps (depending on initial conditions) lead to ten or more openings and about 3% of jumps lead to twenty or more openings.

(ii) *The lengths of openings*

With only one open state, the open-time distributions, given by (2.22), (2.24) and (2.27), and whatever the initial conditions, all reduce to the simple exponential distribution

$$f(t) = (1/\tau)e^{-t/\tau}, \quad t \geq 0, \tag{5.7}$$

with time constant $\tau = -1/q_{11} = 1.092$ ms.

Table 2. Probability distribution of the number of openings

r	shut initially	open initially	overall
0	0.1684	0	0.1669
1	0.1398	0.1682	0.1401
2	0.1163	0.1399	0.1165
3	0.0968	0.1164	0.0969
4	0.0805	0.0968	0.0806
5	0.0670	0.0805	0.0671
≥ 10	0.1586	0.1907	0.1589
≥ 20	0.0252	0.0303	0.0252

(iii) *Shut times within bursts*

Shut times within a burst all follow a sojourn in the single open state, so the distributions (2.30), (2.32) and (2.36), regardless of r , n or the initial state of the channel, all reduce to the same distribution. From the spectral expansion of $\exp(\mathbf{Q}_{BB}t)$, see Colquhoun & Hawkes (1995*b*), this is a mixture of two exponentials,

$$f(t) = (a_1/\tau_1)e^{-t/\tau_1} + (a_2/\tau_2)e^{-t/\tau_2}, \quad (5.8)$$

where the time constants, $\tau_1 = 15.49$ ms and $\tau_2 = 641.4$ ms, are the negative reciprocals of the eigenvalues of \mathbf{Q}_{BB} and have corresponding areas $a_1 = 0.8628$ and $a_2 = 0.1372$. The mean shut time is 101.4 ms.

(iv) *The first latency distribution*

If the channel is shut initially, the latency to the first opening, assuming there is one, again consists of a sojourn in the \mathcal{B} states and therefore also has the form (5.8), with the same time constants. However, whereas a shut time within a burst of openings must start in state 3 (A_2R), the first latency is more likely to start in state 2 (A_2D), as is clear from the equilibrium occupancies in (5.4). The areas are now $a_1 = 0.1319$ and $a_2 = 0.8681$, so the slow component predominates (because of the long mean lifetime of state 2) and the mean is 558.9 ms. Again, because of the lack of correlation in this mechanism, this distribution is obtained from (2.39) or from (2.38), regardless of the value of r . It is illustrated in figure 4*a*.

(v) *The burst length and the length of the whole activation*

The burst of openings, if there is one, after a jump consists of a sojourn in the set of states $\mathcal{E} = \mathcal{A} \cup \mathcal{B}$. We see from (2.41) that the probability distribution of its length will be a mixture of three exponentials, the time constants being the negative reciprocals of the eigenvalues of $\mathbf{Q}_{\mathcal{E}\mathcal{E}}$. Once again, the fact that there is just one open state means that we get the same distribution regardless of the conditions at time zero. The time constants and corresponding areas are given in table 3; the mean burst length is 508 ms. The distribution is illustrated in figure 4*b*.

As remarked in §2, the total activation time is the same as the burst length if the channel is open at time zero. If the channel is shut at time zero, the total activation is also a sojourn in the set of states \mathcal{E} , but starting in \mathcal{B} rather than \mathcal{A} . The distribution is therefore also a mixture of the same three exponentials, but with different areas. These are also shown in table 3. We note that, in this case, one of the areas is

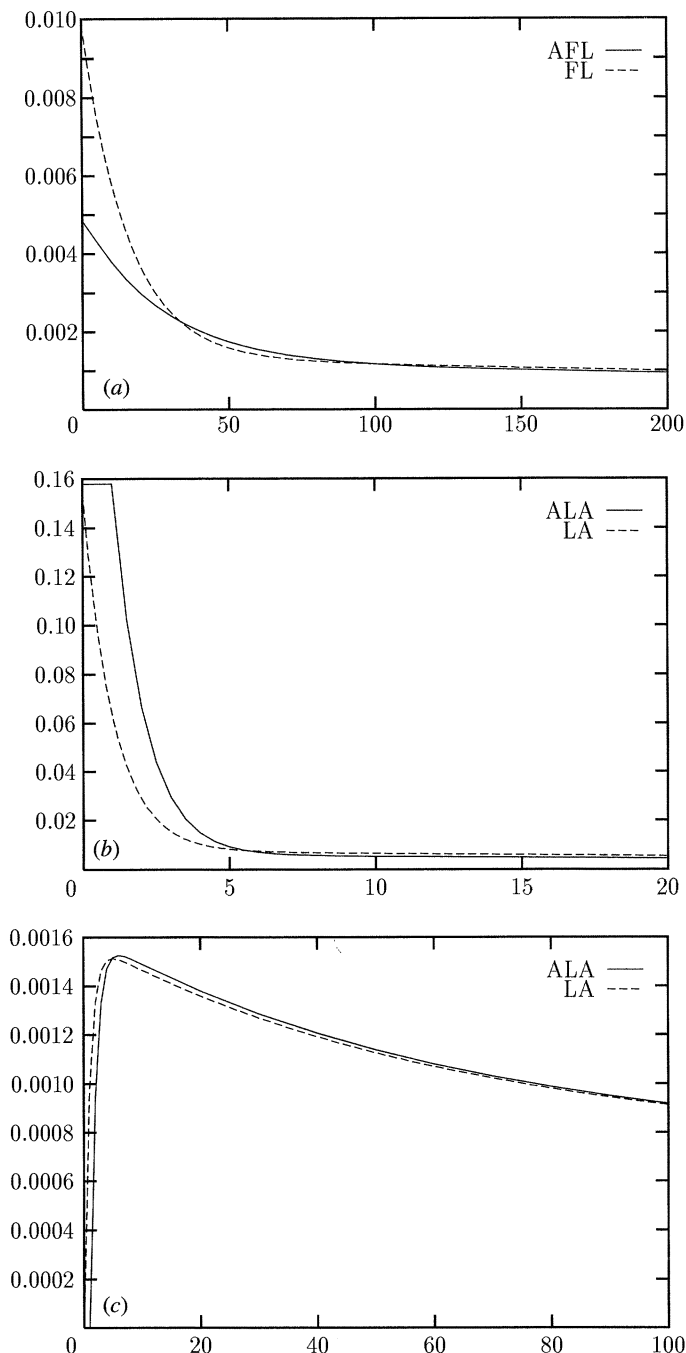


Figure 4. Distributions following a jump to zero concentration. (a) shows the distributions of first latency (FL) and apparent first latency (AFL), the latter allowing for time interval omission with a deadtime of 1 ms; (b) shows the distributions of total length of activation (LA), given that the channel is open initially, and apparent length of activation (ALA), given that the channel is apparently open initially (this is the same as the distribution of burst length); (c) shows the distributions of length of activation (LA) and apparent length of activation (ALA) conditional on the channel being shut (or apparently shut) initially.

Table 3. Distributions for total activation and burst length after a jump to zero concentration

time constants	length of activation			burst length
	area when channel shut initially	area when channel open initially	overall distribution	
1.038 ms	-0.0016	0.1519	-0.00001	0.1519
56.11 ms	0.0406	0.4105	0.04456	0.4105
1108 ms	0.9610	0.4376	0.95545	0.4376
mean sojourn	1067 ms	508 ms	1061 ms	508 ms

negative and the probability density is zero at the origin, i.e. $f(0) = 0$, because in this situation at least two transitions are needed to complete an activation. It is illustrated in figure 4c. The overall distribution of activation time is a mixture of these two distributions with weights $P(\mathcal{A})/P(R \geq 1)$ and $P(\mathcal{F}) \times P(R \geq 1|\mathcal{F})/P(R \geq 1)$.

(vi) *The total open time per burst*

From (2.50), we find that V_{AA} is the 1×1 matrix $[-0.15403]$, so that the distribution of the total open time in a burst, conditional on their being one, has the form of a single exponential with mean $1/0.15403 = 6.49$ ms. This is also equal to the mean number of openings, 5.947 from table 1, multiplied by the mean open time, 1.092.

(vii) *The relationship with channel behaviour in the steady state*

At low agonist concentrations, channel openings recorded in the steady state will occur in well defined bursts, which consist of periods spent in set \mathcal{E} . Since, in this case, there is only one open state, the properties of the burst following the initial opening are always the same and the distribution of the burst length in the steady state will be as in table 3, with a mean burst length of 508 ms and a mean of 5.947 openings per burst (as in table 1). For example, at a concentration of $x = 0.01 \mu\text{M}$, such bursts will be separated by a mean interburst shut time (i.e. a shut period containing at least one sojourn in the set \mathcal{C}) of 1154 s and so will be easily discernible on the experimental record.

(viii) *The relationship with macroscopic channel behaviour*

In general, the macroscopic time course of the response to a jump will have $k - 1$ exponential components with time constants obtained from the (non-zero) eigenvalues of \mathbf{Q} . However, as discussed above, following (2.47), in the case of a jump to zero agonist concentration, the eigenvalues of \mathbf{Q} itself will be the same as the $k_{\mathcal{E}}$ eigenvalues of $\mathbf{Q}_{\mathcal{E}\mathcal{E}}$, plus the $k_{\mathcal{C}}$ eigenvalues of $\mathbf{Q}_{\mathcal{C}\mathcal{C}}$. The time constants that correspond to the eigenvalues of $\mathbf{Q}_{\mathcal{E}\mathcal{E}}$ are given in tables 3 and 4. They are the time constants for both the burst length and activation length at zero concentration after a step to zero (table 3), or at the end of a pulse (table 4). As expected, the time constants for the macroscopic time course are the same as these, plus $\tau_4 = 212.77$ ms, which is the negative reciprocal of the non-zero eigenvalue of $\mathbf{Q}_{\mathcal{C}\mathcal{C}}$. This last component, however, has a zero amplitude in the macroscopic relaxation, which is therefore described by three ($k_{\mathcal{E}}$) exponential components with time constants of 1.038, 56.11 and 1108 ms.

The areas or amplitudes corresponding to these components can be obtained from the spectral expansion of either (2.47) or (2.48).

If we compare (2.48) with (2.45), we see that, in general, the macroscopic time course is different from the activation length distribution. However, if, as in our example, there is only one open state then the terms which are different in the two expressions (namely $(-Q_{AA})e_b/P(R \geq 1)$ and $N\gamma u_A$) are scalars; thus the two expressions are proportional to each other. This means that, following a jump to zero concentration, after equilibration with $x = 1$ mM, the *areas* (i.e. the charge passed) under each exponential component of the macroscopic relaxation are proportional to the areas under the overall distribution of the activation length given in table 3 (e.g. 95.5% of the charge is accounted for by the slowest component). For the step to zero concentration following a 50 ms pulse to 1 mM (see below), the macroscopic current relaxes with the same time constants and the areas for each component are proportional to the areas under the overall distribution of the activation length given in table 4 (e.g. 68.7% of the charge is accounted for by the slowest component).

Notice that the burst length distribution, which is all that can be inferred from low-concentration steady state records, does *not* give a good indication of what the macroscopic relaxation will look like. This is because, in this example, the first latency can be quite long, so its contribution to the activation length cannot be ignored. In cases where the first latency is very short (as for acetylcholine-activated channels at the neuromuscular junction), the burst length measured in low-concentration steady state records gives a good indication of the appearance of macroscopic relaxations. This question is considered further in Colquhoun & Hawkes (1995a).

(ix) *The effect of time interval omission*

As mentioned at the end of §4, the theory allowing for time interval omission is quite complicated for the jump from non-zero concentration. We therefore give no details, but some results have been calculated using the methods of Merlushkin & Hawkes (1995b) and are presented graphically in figure 4. We will assume a deadtime of $\xi = 1$ ms which we have chosen to be quite long, compared with what is normally experimentally possible, so as to emphasize the difference in the results compared with the ideal ($\xi = 0$) theory that we have already dealt with. The initial conditions may be partially summarized by saying that the probability of the channel being 'apparently shut' at $t = 0$ is 0.99308 and that of being 'apparently open' is 0.00692. Figure 4a shows the distribution of apparent first latency assuming the channel is apparently shut at $t = 0$ and that there is at least one subsequent apparent opening. Compared with the results without time interval omission there is a relative lack of short latencies, presumably due to missing some short openings early on.

The distribution of total apparent length of activation conditional on the channel being apparently open initially is shown in figure 4b. This distribution has quite a few more activations of less than 5 ms, compared with the ideal case; this is presumably due to apparent premature termination of the activation because of missing subsequent short open times after the first one. The shape is also interesting, being almost uniform between 0 and ξ , then decaying rapidly. The first apparent opening must exceed ξ , but only that portion which occurs after $t = 0$ is recorded, so this early part is (almost) what one would expect to get from a classical recurrence time distribution. In this case, apparent length of activation is the same as apparent burst length, so the distribution is the same; but note that, unlike the ideal case, the apparent burst length distribution will be different if the channel is apparently

shut initially because, for example, the apparent burst must then be of at least ξ in duration.

Figure 4c shows the distribution of apparent length of activation, conditional on being apparently shut initially and having at least one apparent opening. The results do not differ much from the ideal case, but there is some loss of short activations. This is because some short actual activations will contain just one or two short open times which may not be observed because of time interval omission, so there is apparently no response at all to the jump: remember, this distribution is conditional on observing at least one open time. Another reason is that the apparent length of an activation must be at least ξ if the channel is apparently shut at time $t = 0$.

(b) *Response to a pulse of agonist—recording from the end of the pulse*

We now suppose that a pulse of agonist is applied for a period of 50 ms. We have made this quite long so as to emphasize the within-pulse behaviour, which we do later—here we are concerned with what happens after the pulse. The transition rate matrix applying during the pulse is Q^1 , from (5.2), while the transition rate matrix Q^0 , from (5.3), applies both before and after the pulse. We present results without time interval omission only.

If zero concentration has been held for long enough before the pulse for the channel to reach equilibrium, the initial vector in equation (3.1) can be taken as $\mathbf{p}(0) = [0 \ 0 \ 0 \ 0 \ 1]$, giving a new initial vector $\mathbf{p}(0) \equiv \mathbf{p}(T)$ at the end of the pulse. This is

$$\mathbf{p}(0) = [0.03332 \mid 0.31426 \quad 0.65119 \mid 0.00123 \quad 5.76 \times 10^{-7}]. \quad (5.9)$$

Clearly, the pulse of agonist is not long enough to reach equilibrium under the Q^1 matrix regime. Compared with (5.4), the channel is more likely to be found in the open state, or in the set \mathcal{C} ; the most notable difference, however, is that within the set \mathcal{B} it is now much more likely to be found in state 3 (A_2R) rather than state 2 (A_2D).

When we record from the end of the pulse, it is equivalent to a single jump using this modified initial vector, so the theory of § 2 still applies. The transition matrices given in (5.5) remain unaltered and the special nature of G_{BA} and G_{BC} , having identical rows, means that the big change in the initial probability of state 2 versus state 3 makes no difference at all to the distribution of the number of openings. The other relatively minor changes in $\mathbf{p}(0)$ alter probabilities in tables 1 and 2 by at most 3 in the third decimal place, while the means change by at most 2 in the second decimal place (we do not, therefore, present details). The probabilities and mean conditional on the channel being open initially are, of course, unaltered ('initially' now means 'at the end of the pulse').

Because there is only one open state, this is true of the distribution of any characteristic which depends only on behaviour following the first opening. Thus the distributions of open times, shut times within bursts, burst length and total open time per burst are all identical with those given previously.

(i) *First latency and length of activation*

The big change in the initial probabilities does have a significant effect on the distribution of first latency and the total activation time, which includes it. When the channel is shut initially, the distribution of first latency still has the form of a mixture of two exponentials with time constants 15.49 ms and 641.4 ms, but the corresponding areas are now 0.5739 and 0.4261. Compared with the first latency

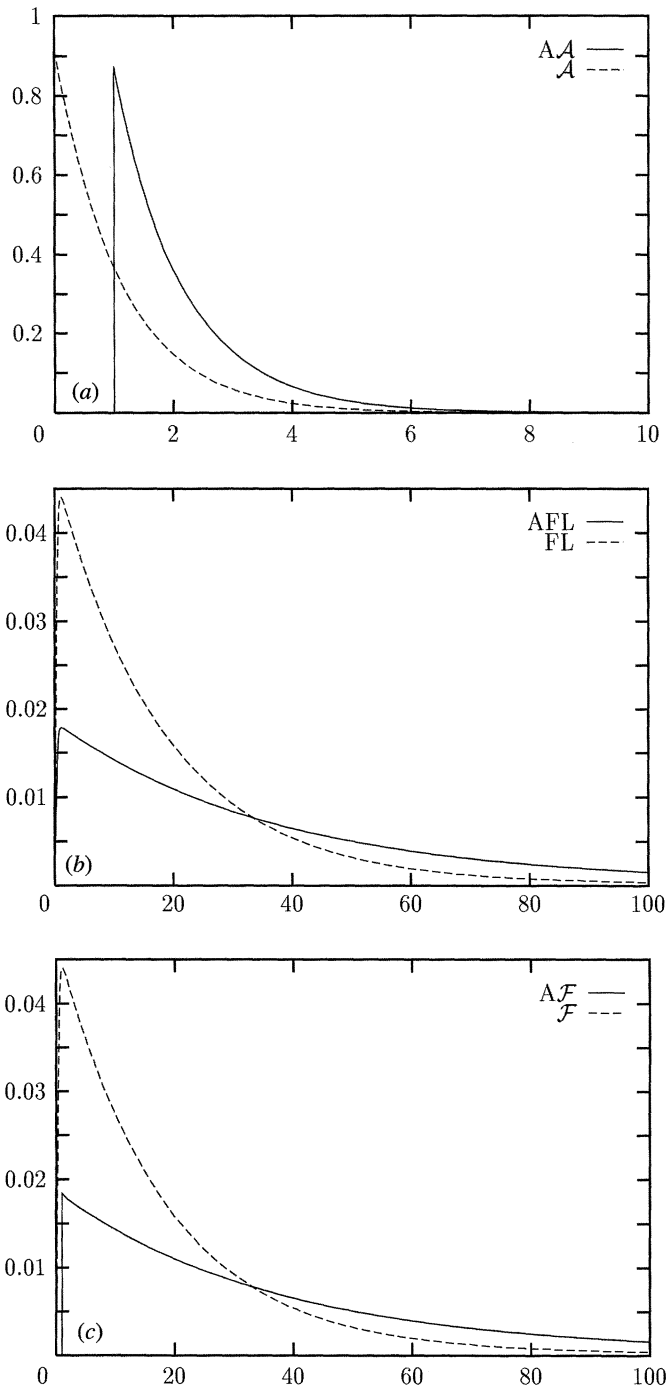


Figure 5. Distributions following a jump from zero concentration. (a) shows the distributions of open times (\mathcal{A}) and apparent open times ($A\mathcal{A}$), the latter allowing for time interval omission with a deadtime of 1 ms; (b) shows the distributions of first latency (FL) and apparent first latency (AFL); (c) shows the distributions of shut times (other than the first latency), the actual distribution (\mathcal{F}) and the apparent distribution ($A\mathcal{F}$).

Table 4. Distributions of the length of activations after a pulse

time constants	area when channel shut initially	overall distribution
1.038 ms	-0.0064	-0.00006
56.11 ms	0.3087	0.31273
1108 ms	0.6977	0.68733
mean sojourn	790 ms	779 ms

distribution discussed earlier, this represents a big shift to the faster component with a resulting reduction in the mean to 282.2 ms.

The new results for the distribution of total activation time are given in table 4. The time constants are the same as in table 3, but the areas and means are very different. As with first latency, there is a reduction in mean, because there is a smaller chance of spending a long initial period in state 2 (A_2D). When the channel is open initially, the length of the activation is the same as the burst length so that, as stated above, there is no change in that distribution.

(c) *Single jump from zero concentration*

We now suppose that there is zero agonist concentration before time zero, at which point it jumps to a concentration of 1 mM. We take the initial probability to be the equilibrium vector of the matrix \mathbf{Q}^0 , i.e. $\mathbf{p}(0) = [0\ 0\ 0\ 0\ 1]$, so the channel is certain to be in state 5. The transition rate matrix \mathbf{Q}^1 then applies after the jump. The theory allowing for time interval omission is given in equations (4.9)–(4.13). As before, we assume a deadtime $\xi = 1$ ms. The results for the ideal theory, $\xi = 0$, are easily found as a special case of the above equations.

With only one open state, the true open time distribution is a simple exponential with mean 1.09 ms, exactly the same as for the jump to zero concentration discussed earlier. Apparent open times, allowing for time interval omission, have a mean of 1.167 ms. The distribution is no longer of simple exponential form but, for $t > 3\xi$, it is well approximated by

$$f(t) \approx a(1/\tau)e^{-(t-\xi)/\tau}, \quad (5.10)$$

with time constant $\tau = 1.175$ and ‘area’ $a = 0.9913$. These two distributions are shown in figure 5a. Note that the apparent open time is always greater than ξ , but the shape of the distribution is otherwise not that much different (because shut times are not often missed).

The true shut time distributions are governed, essentially, by the function $\exp(\mathbf{Q}_{\mathcal{F}\mathcal{F}}^1 t)$. The spectral expansion of this implies that they must have the form of mixtures of four exponentials with time constants the negative reciprocals of the eigenvalues of $\mathbf{Q}_{\mathcal{F}\mathcal{F}}^1$. Because of the lack of correlation in this simple mechanism, all shut times following the first opening have the same distribution, which is different from that of the first latency.

The distribution of apparent first latency is not a mixture of exponentials. From (4.9), we see that it depends on $\mathcal{F}\mathbf{R}(t)$, the asymptotic expansion of which (see Appendix) means that, for $t > 2\xi$, the distribution can be well approximated by a

Table 5. Areas for exponential components of shut times after a jump from zero concentration (Areas and time constants for the apparent distributions relate to the asymptotic approximations in (5.11)–(5.12); results for the actual distributions are exact)

actual ($\xi = 0$)			apparent ($\xi = 1$ ms)		
τ (ms)	first latency a	other shuttings a	τ (ms)	first latency a	other shuttings a
0.100	0.0047	4.1×10^{-9}	0.100	0.0004	-2.5×10^{-10}
0.200	-0.0188	1.8×10^{-5}	0.200	-0.0067	-6.5×10^{-7}
18.16	0.8522	0.8328	36.73	0.6642	0.6528
659.3	0.1619	0.1618	824.1	0.3412	0.3471
mean	122.2 ms	121.9 ms	mean	305.6 ms	311.1 ms

mixture of four exponentials

$$f(t) \approx \sum_{i=1}^4 a_i(1/\tau_i)e^{-t/\tau_i}. \tag{5.11}$$

The distributions of true and apparent first latencies are compared in figure 5*b*.

Unlike the apparent first latency, all apparent shut times after the apparent first opening must exceed ξ in duration, see discussion following (4.13). Their distribution depends on ${}^{\mathcal{F}}R(t-\xi)$, so the asymptotic expansion of this which is used for $u = t-\xi > 2\xi$ means that, for $t > 3\xi$, the shut time distribution from (4.13) is well approximated by

$$f(t) \approx \sum_{i=1}^4 a_i(1/\tau_i)e^{-(t-\xi)/\tau_i}, \tag{5.12}$$

where the time constants are the same as in (5.11) but the areas, a_i , are different.

The lack of correlation in this example implies that this distribution is the same for all n .

The distributions of true and apparent shut times (other than the first latency) are compared in figure 5*c*. The time constants and areas discussed above are given in table 5, together with means.

Although there are four components, two of them are rather fast and have negligible areas, except for the first latency where they modify the behaviour for small t . Apart from that there is not very much difference between the distribution of first latency and that of other shut times, compare figures 5*b* and 5*c*. There is, however, a big difference between the distributions of real and apparent shut times, with means of about 120 and 310 ms, respectively. This is clearly mainly due to missing a few short open times, so that the neighbouring shut times get concatenated to create a long apparent shut time. Thus, the two slow asymptotic time constants of the apparent distribution are somewhat larger than those of the actual distribution, but the two short ones are identical, to the degree of accuracy recorded.

Note that, except for first latency, the distributions obtained here (actual or apparent) are the same as would be obtained from equilibrium recording under the transition matrix Q^1 . This is due to the lack of correlation in this mechanism.

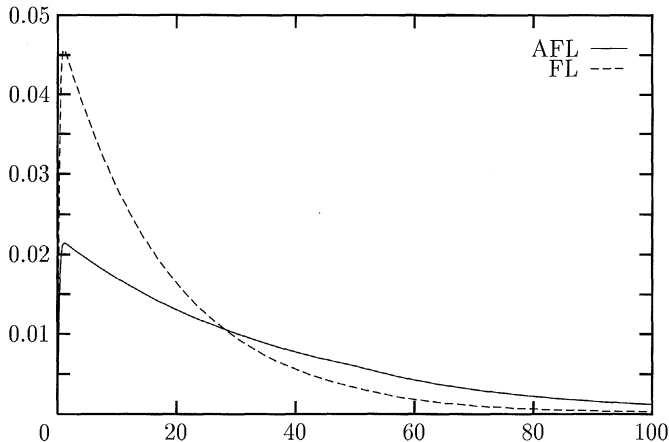


Figure 6. Distributions of first latency (FL) and apparent first latency (AFL), deadtime $\xi = 1$ ms, in response to a 50 ms pulse of agonist.

(d) *Response to a pulse: recording from the beginning of the pulse*

As before, we consider a 50 ms pulse during which agonist concentration is 1 mM and so transition rate matrix Q^1 applies. The transition rate matrix Q^0 applies before and after the pulse, when zero concentration obtains, and the initial probability vector is $\mathbf{p}(0) = [0 \ 0 \ 0 \ 0 \ 1]$, so the channel is known to be shut initially. This time we start recording from the beginning of the pulse instead of the end, so that within-pulse activity can be studied. The theory incorporating time interval omission, again taking $\xi = 1$ ms, is given in equations (4.1)–(4.8) and the ideal theory, when $\xi = 0$, is dealt with in equations (3.2)–(3.11) and (3.14)–(3.27).

The probabilities of displaying at least one opening, or at least one apparent opening, after $t = 0$ are

$$P(R \geq 1) = 0.96553 \quad \text{and} \quad P(\hat{R} \geq 1) = 0.83472,$$

respectively, so about 13% of bursts of activity would be missed because of time interval omission.

The distributions of first latency and apparent first latency are shown in figure 6. Because there is quite a high probability of the first opening occurring before the end of the pulse (i.e. before 50 ms), they are quite similar to what happens in the case of a single jump from zero concentration (compare with figure 5b).

Figure 7a shows the distributions of length of activation and apparent length of activation. Clearly there is a dramatic difference in behaviour before and after the end of the pulse. The distribution of apparent length of activation is shifted towards the origin, compared with the ideal case, because of missing some openings in the tail end of the true activation. As these distributions look quite strange, and the mathematics is quite complicated, we checked them by simulation. The results in figure 7b show that the simulated and theoretical distributions compare well.

Finally, the distribution of burst length, in the ideal case, is shown in figure 8. The steep initial part reflects the contribution when a burst consists of a single opening. If there are two or more openings, the burst is considerably lengthened, because the shut times tend to be relatively long, and the curve rises then tails away as it passes the end of the pulse (50 ms). The burst length allowing for time interval omission is even more complicated and has not been attempted.

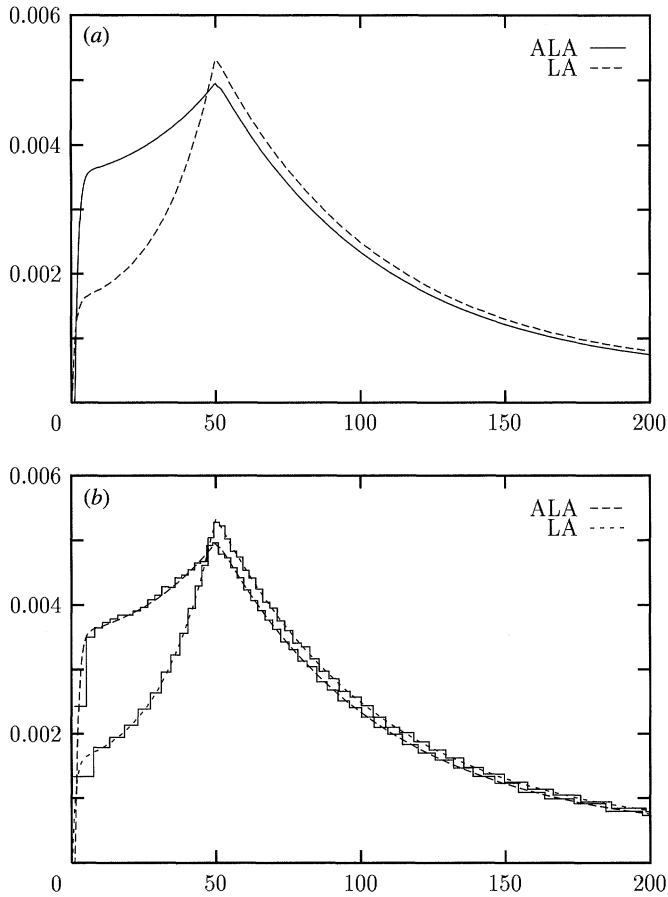


Figure 7. Distributions of length of activation (LA) and apparent length of activation (ALA), deadtime $\xi = 1$ ms, in response to a 50 ms pulse of agonist. (a) shows the theoretical distributions; (b) shows the results of simulation superimposed on the theoretical curves.

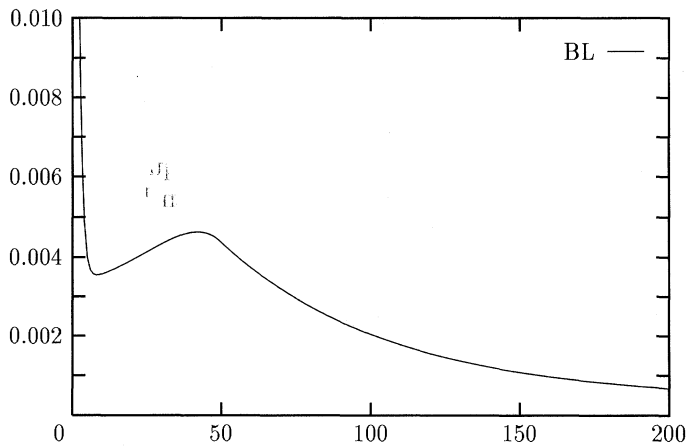


Figure 8. Distribution of actual burst lengths in response to a 50 ms pulse of agonist.

6. Discussion

In all but the simplest cases, it is unlikely that single channel records at equilibrium will contain enough information to identify a reasonable mechanism. Exploiting the extra information that is present in non-stationary recordings can provide valuable insights. It will often, therefore, be important to measure the properties of macroscopic currents, and especially single channels, that are elicited by a step, or a pulse, of agonist concentration (or any other variable that affects channel activity).

Channels that are present in synapses will, in real life, never be in a steady state during synaptic transmission when they are exposed only briefly to a pulse of agonist concentration. The time course of the agonist concentration is hard to determine precisely, but it is not likely to take the form of the rectangular pulse which is assumed in §3. However, the characteristics of channels that are elicited by a brief pulse are unlikely to be much influenced by the exact shape of the pulse when the pulse is brief relative to the (average) duration of the events that it elicits, since the concentration will be zero for most of the time during the recording. This is a rationale for the attempts that have been made to mimic synaptic activity by application of brief rectangular pulses of agonists to ion channels (see Introduction). It is now possible to generate sub-millisecond pulses which are not far from rectangular. The measurement of non-stationary macroscopic currents by this method is now a relatively routine business. This approach is usually the only way to measure single channel activity at zero concentration, since at equilibrium there will (in most cases) be no openings to measure.

We note that an interesting result to emerge from our numerical example is that the time course of the macroscopic current following a jump to zero concentration is proportional to the probability density of the total activation time, in the case where there is only one open state.

The biggest practical problem is still the fact that in order to measure quantities such as the latency to the first channel opening, it is necessary to know the exact number of channels in a membrane patch, and preferably there should be only one (as is assumed throughout this paper). This is hard to achieve in practice, but it is not impossible (see Wyllie *et al.* 1997).

The most obvious quantity to measure is the latency from the moment at which the jump, or pulse, is applied to the time when a channel first opens. Indeed, for mechanisms that show no correlations (see Fredkin *et al.* 1985; Colquhoun & Hawkes 1987), this may be the *only* thing that will differ from equilibrium recordings. In the case, for example, of a single jump to a finite agonist concentration, all the other sorts of distributions that are listed here (apart from the total activation length which includes the first latency) will be the same as in equilibrium recordings (made at the post-jump concentration). For example, the mean length of the n th opening after a jump will be the same (the equilibrium value) for any n if there are no correlations (e.g. Colquhoun & Hawkes 1987). The same is likely to be true, to a good approximation, for very brief pulses, for which the structure of the burst of openings elicited by the pulse is expected to be very similar to the burst structure recorded at equilibrium in very low agonist concentrations (low enough that the channel is close to the limiting behaviour expected as concentration approaches the post-pulse value of zero).

In reality, however, most channels do seem to show correlations, in cases where it has been measured (e.g. Colquhoun & Sakmann 1985; Ball *et al.* 1989; Gibb & Colquhoun 1992). In this case, all the distributions that are listed here will provide

useful information about the nature of the mechanism. This information is related to, but not identical with, that found by measuring correlations or conditional distributions in equilibrium records (e.g. Fredkin *et al.* 1985; McManus *et al.* 1985; Colquhoun & Hawkes 1987; Ball *et al.* 1989; Colquhoun *et al.* 1996).

In this paper, we have given general solutions to the problems of single jumps and pulses, with particular attention to the problems that arise when predicting the consequences of a jump to zero agonist concentration. In the ideal case of perfect time resolution, the distributions can be found by similar methods to those used for equilibrium records (see, for example, Colquhoun & Hawkes 1995*b*) and most of them are no more difficult. An exception is the distribution of the length of the burst of openings which follows a pulse; even in the ideal case this gets a bit more complicated than any equilibrium distribution, as shown in (3.19)–(3.27).

Some of the relevant problems can also be solved with exact allowance for the limited resolution of the recording system, as outlined in §4. However, the results get a good deal more complicated in this case and here we have discussed only the simplest cases, the distributions of the apparent first latency, the probability of observing at least one apparent opening and the distribution of the apparent length of the whole activation. See Merlushkin & Hawkes (1995*a*) for details.

The results given here should be useful as a basis for the interpretation of more complex experiments. For example, Colquhoun & Hawkes (1995*c*) considered the interpretation of experiments in which macroscopic responses to a pulse of agonist concentration were measured after pre-exposure to a longer prepulse of a low agonist concentration (Lin & Stevens 1994). The size of the response to the test pulse depended on whether single channel openings were observed during the prepulse or not. In this problem, the probability that there will be no channel openings during the prepulse is just the probability that the first latency following the step to the prepulse concentration is longer than the length of the prepulse and so can be found from the first latency distribution given here (following (3.13)). The treatment of Colquhoun & Hawkes (1995*c*) could be extended to allow for missed events using the approach outlined here: for example, the distribution of apparent first latency given in (4.1) could be integrated numerically to give the probability that there will be no *detectable* channel openings during the prepulse.

A.G.H. acknowledges the support of the Wellcome Trust, which contributed to this work. D.C. was supported by the Medical Research Council and the Wellcome Trust.

Appendix A.

Formulae for the matrix function $\mathcal{F}\mathbf{R}(u)$.

The starting point is the representation of the matrix \mathbf{Q} in terms of the spectral matrices \mathbf{A}_i so that, assuming the matrix $-\mathbf{Q}$ has distinct eigenvalues λ_i ,

$$\exp(\mathbf{Q}u) = \sum_{i=1}^k \mathbf{A}_i \exp(-\lambda_i u). \tag{A 1}$$

Let $\mathbf{A}_{i\mathcal{FA}}$ be the \mathcal{FA} partition of \mathbf{A}_i and define

$$\mathbf{D}_i = \mathbf{A}_{i\mathcal{FA}} \exp(\mathbf{Q}_{\mathcal{AA}}\xi) \mathbf{Q}_{\mathcal{AF}}. \tag{A 2}$$

Then $\mathcal{F}\mathbf{R}(u)$ is given by

$$\mathcal{F}\mathbf{R}(u) = \begin{cases} \mathbf{N}_0(u), & 0 \leq u < \xi, \\ \mathbf{N}_0(u) - \mathbf{N}_1(u - \xi), & \xi \leq u < 2\xi, \end{cases} \tag{A 3}$$

where

$$\mathbf{N}_0(u) = \sum_{i=1}^k \mathbf{C}_{i00} \exp(-\lambda_i u), \quad \mathbf{N}_1(u) = \sum_{i=1}^k (\mathbf{C}_{i10} + \mathbf{C}_{i11} u) \exp(-\lambda_i u) \tag{A 4}$$

and the matrices \mathbf{C}_{iml} are given recursively by

$$\mathbf{C}_{i00} = \mathbf{A}_{i\mathcal{F}\mathcal{F}}, \quad \mathbf{C}_{i10} = \sum_{j \neq i} (\mathbf{D}_i \mathbf{C}_{j00} + \mathbf{D}_j \mathbf{C}_{i00}) / (\lambda_j - \lambda_i), \quad \mathbf{C}_{i11} = \mathbf{D}_i \mathbf{C}_{i00}. \tag{A 5}$$

Approximate evaluation of $\mathcal{F}\mathbf{R}(u)$ for large u .

These results can be extended for values of $u \geq 2\xi$, but we use instead an asymptotic form which has been found in practice to be very accurate (even for smaller values of u than this, in some cases). The asymptotic behaviour of $\mathcal{F}\mathbf{R}(u)$ depends on the values of s which render singular the matrix $\mathbf{W}(s)$ defined as

$$\mathbf{W}(s) = s\mathbf{I} - \mathbf{H}(s), \tag{A 6}$$

where

$$\mathbf{H}(s) = \mathbf{Q}_{\mathcal{F}\mathcal{F}} + \mathbf{Q}_{\mathcal{F}\mathcal{A}}(s\mathbf{I} - \mathbf{Q}_{\mathcal{A}\mathcal{A}})^{-1} \mathbf{S}_{\mathcal{A}\mathcal{A}}^*(s) \mathbf{Q}_{\mathcal{A}\mathcal{F}} \tag{A 7}$$

and

$$\mathbf{S}_{\mathcal{A}\mathcal{A}}^*(s) = \mathbf{I} - \exp(-(s\mathbf{I} - \mathbf{Q}_{\mathcal{A}\mathcal{A}})\xi), \tag{A 8}$$

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

$$\det[\mathbf{W}(s)] = 0. \tag{A 9}$$

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

$$\mathcal{F}\mathbf{R}(u) \sim \sum_{i=1}^{k_{\mathcal{F}}} \mathcal{F}\mathbf{R}_i e^{-u/\tau_i}, \tag{A 10}$$

where

$$\tau_i = -1/s_i, \quad \mathcal{F}\mathbf{R}_i = \mathbf{c}_i \mathbf{r}_i / \mathbf{r}_i \mathbf{W}'(s_i) \mathbf{c}_i \tag{A 11}$$

and $\mathbf{c}_i, \mathbf{r}_i$ are the right and left (column and row) eigenvectors of $\mathbf{H}(s_i)$ corresponding to the root s_i . The matrix derivative in the above results can be evaluated as

$$\mathbf{W}'(s) = \mathbf{I} + \mathbf{Q}_{\mathcal{F}\mathcal{A}} [\mathbf{S}_{\mathcal{A}\mathcal{A}}^*(s)(s\mathbf{I} - \mathbf{Q}_{\mathcal{A}\mathcal{A}})^{-1} - \xi(\mathbf{I} - \mathbf{S}_{\mathcal{A}\mathcal{A}}^*(s))] \mathbf{G}_{\mathcal{A}\mathcal{F}}^*(s), \tag{A 12}$$

where $\mathbf{G}_{\mathcal{A}\mathcal{F}}^*(s)$ is given in equations (1.5).

Laplace transform of $\mathcal{F}\mathbf{R}(u)$.

Hawkes *et al.* (1990) showed that the Laplace transform of $\mathcal{F}\mathbf{R}(u)$, $\mathcal{F}\mathbf{R}^*(s)$ is given by

$$\mathcal{F}\mathbf{R}^*(s) = [\mathbf{V}_{\mathcal{F}}^*(s)]^{-1} [s\mathbf{I} - \mathbf{Q}_{\mathcal{F}\mathcal{F}}]^{-1}, \tag{A 13}$$

where

$$\mathbf{V}_{\mathcal{F}}^*(s) = \mathbf{I} - \mathbf{G}_{\mathcal{F}\mathcal{A}}^*(s) \mathbf{S}_{\mathcal{A}\mathcal{A}}^*(s) \mathbf{G}_{\mathcal{A}\mathcal{F}}^*(s). \tag{A 14}$$

Distributions of apparent shut times under a homogeneous regime with transition rate matrix \mathbf{Q} are determined by the semi-Markov matrix function of probability densities

$${}^e\mathbf{G}_{\mathcal{F}\mathcal{A}}(t) = {}^{\mathcal{F}}\mathbf{R}(t - \xi)\mathbf{Q}_{\mathcal{F}\mathcal{A}} \exp(\mathbf{Q}_{\mathcal{A}\mathcal{A}}\xi); \tag{A 15}$$

compare this with (4.10). This is both pre-multiplied and post-multiplied by appropriate vectors to obtain a probability density; see, for example, (4.12). ${}^e\mathbf{G}_{\mathcal{F}\mathcal{A}}(t)$ has Laplace transform

$${}^e\mathbf{G}_{\mathcal{F}\mathcal{A}}^*(s) = {}^{\mathcal{F}}\mathbf{R}^*(s)\mathbf{Q}_{\mathcal{F}\mathcal{A}} \exp(-(s\mathbf{I} - \mathbf{Q}_{\mathcal{A}\mathcal{A}})\xi). \tag{A 16}$$

To obtain the corresponding mean apparent shut time, we simply replace the matrix function ${}^e\mathbf{G}_{\mathcal{F}\mathcal{A}}(t)$ in the formula for the density by the constant matrix $\mathbf{M}_{\mathcal{F}\mathcal{A}}$, which is given by

$$\mathbf{M}_{\mathcal{F}\mathcal{A}} = \left[-\frac{d}{ds} {}^e\mathbf{G}_{\mathcal{F}\mathcal{A}}^*(s) \right]_{s=0} = \xi {}^e\mathbf{G}_{\mathcal{F}\mathcal{A}} + \left[-\frac{d}{ds} {}^{\mathcal{F}}\mathbf{R}^*(s) \right]_{s=0} \mathbf{Q}_{\mathcal{F}\mathcal{A}} \exp(\mathbf{Q}_{\mathcal{A}\mathcal{A}}\xi), \tag{A 17}$$

where

$$\left[-\frac{d}{ds} {}^{\mathcal{F}}\mathbf{R}^*(s) \right]_{s=0} = \mathbf{V}_{\mathcal{F}}^{-1}\mathbf{Q}_{\mathcal{F}\mathcal{F}}^{-2} + \mathbf{V}_{\mathcal{F}}^{-1} \left[-\frac{d}{ds} \mathbf{V}_{\mathcal{F}}^*(s) \right]_{s=0} \mathbf{V}_{\mathcal{F}}^{-1}\mathbf{Q}_{\mathcal{F}\mathcal{F}}^{-1} \tag{A 18}$$

and

$$\left[-\frac{d}{ds} \mathbf{V}_{\mathcal{F}}^*(s) \right]_{s=0} = \mathbf{Q}_{\mathcal{F}\mathcal{F}}^{-1}\mathbf{G}_{\mathcal{F}\mathcal{A}}\mathbf{S}_{\mathcal{A}\mathcal{A}}\mathbf{G}_{\mathcal{A}\mathcal{F}} + \mathbf{G}_{\mathcal{F}\mathcal{A}}\mathbf{S}_{\mathcal{A}\mathcal{A}}\mathbf{Q}_{\mathcal{A}\mathcal{A}}^{-1}\mathbf{G}_{\mathcal{A}\mathcal{F}} + \xi\mathbf{G}_{\mathcal{F}\mathcal{A}} \exp(\mathbf{Q}_{\mathcal{A}\mathcal{A}}\xi)\mathbf{G}_{\mathcal{A}\mathcal{F}}. \tag{A 19}$$

The results (A 17)–(A 19) are, *mutatis mutandis*, essentially those of Colquhoun *et al.* (1996, equation (3.6)). Here, as elsewhere, we employ the simplifying notational convention of removing the asterisk when setting the Laplace parameter to zero so that, for example,

$$\mathbf{S}_{\mathcal{A}\mathcal{A}} = \mathbf{S}_{\mathcal{A}\mathcal{A}}^*(0).$$

Similar results may be obtained for apparent open times by reversing the roles of \mathcal{F} and \mathcal{A} . These results are used at the end of § 4 to obtain matrices $\mathbf{M}_{\mathcal{F}\mathcal{A}}^1$ and $\mathbf{M}_{\mathcal{A}\mathcal{F}}^1$, which are calculated as described here, but the superscript 1 denotes that everything is calculated with respect to the particular transition rate matrix \mathbf{Q}^1 in place of \mathbf{Q} .

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Received 4 June 1996; accepted 17 September 1996