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Stochastic properties of ion channel openings and bursts in a membrane patch that contains two channels: evidence concerning the number of channels present when a record containing only single openings is observed

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If a single ion channel record is observed in which two ion channels are never simultaneously open, then it is often of interest to know whether the observations indeed arose from the activity of only one ion channel. This question can be answered if it is possible to calculate the distribution of the duration of runs of single openings in a membrane patch that contains two active channels. If the observed run of single openings is much longer than that expected for a patch with two channels it is likely that only one channel was active.

An approximate method is presented for calculating the distribution of the duration of runs of single openings in a patch with two active channels; this method has the advantage that it can be calculated from observable quantities, and requires no knowledge of the details of the ion-channel mechanism or its rate constants. The accuracy of this approximation is tested by exact calculations of the properties of runs of single openings, and of single bursts, for two specific mechanisms and a large range of rate constants. The approximation is good in all cases in which openings occur singly, or in closely spaced bursts.

If, as is common in practice, openings occur in clusters that are separated by long shut periods, then overlap of clusters from two different channels may be detected, if no double opening is produced, as a period in the middle of a cluster in which the probability of being open doubles. The results derived here can be applied to such a period to test whether it results from the simultaneous activity of two channels, rather than from a change in the properties of a single channel.

INTRODUCTION

Probably the most serious problem in the interpretation of single ion channel records stems from the fact that the number of channels in the membrane patch from which the recording is made is almost always unknown. However, under conditions where channels are open for a large fraction of the time it may be 'obvious' that only one channel is active if no double openings are seen, because there would be a high probability of two channels opening at the same time if two (or more) channels were active (see, for example, Sakmann *et al.* (1980); Colquhoun & Ogden (1988)). The question frequently arises as to how large a fraction of time the channel must be open, and how long must elapse without seeing a double opening, before we can be confident that only one channel is active.

Suppose the membrane patch contains two identical independent channels, yet only *single openings* are observed, i.e. both channels are never simultaneously open. In this case we have observed a 'run of single openings' with two channels: the problem is to determine the properties of such runs (the definition of a run is illustrated in figure 1, which will be discussed in more detail later). In particular, if the distribution of the length of such runs, and of the number of (single) openings in the runs, can be derived then it will be possible to judge whether the experimentally observed run is so long that it is unlikely that two channels could have been present.

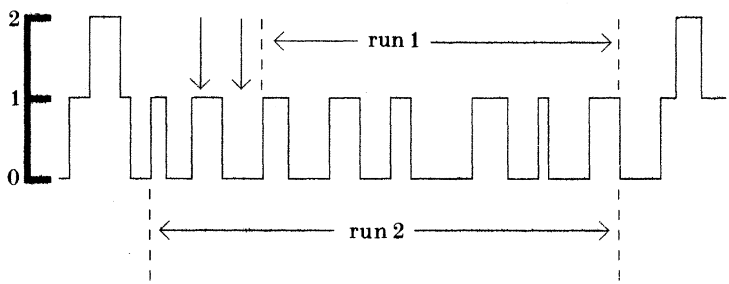

FIGURE 1. Definition of a run of single openings. The scale on the left indicates the number of channels that are open. The entire run of single openings is labelled run 2, but an experimental record would start at some arbitrary time after the double opening. For runs that started at either of the points marked by vertical arrows, the initial vector used for the calculations defines the run of single openings as the section of the record labelled run 1.

Exact calculation of these distributions is possible if the channel mechanism, and the values of all the rate constants it, are known. But in most cases these are not known, and they cannot easily be determined from a two-channel record (according to Yeo *et al.* (1989) this cannot be done unambiguously). However it seems quite likely, from the simple derivation given below, that the length of the run (relative to the length of a single opening), and the number of openings per run, will depend primarily on the P_{open} during the observed run, and will not be very dependent on the underlying model or its rates. We shall now show, by comparison of the approximation with the results of exact calculations, that this conjecture is true under most circumstances. It is, therefore, possible to do a simple test, which requires little knowledge of the channel characteristics, of the hypothesis that only one channel is active. Some exact results for a recording that contains two identical, independent channels will be given.

Simple approximations

The principle of the approximate argument

A useful, but approximate, answer to the problem can be obtained without specifying any model or rate constants. The nature of the argument can be illustrated by an example. Suppose that there are actually two active channels, but no double openings have been observed. The channel is observed to be open for, say, 10% of the time in the record, and the mean open time is 1 ms, so the mean shut time is 9 ms. Each individual channel is thus open for (approximately) 5% of the time ($P_{\text{open}} = 0.05$), with a mean open time of 1 ms, and a mean shut time of 19 ms. The fraction of time occupied by double openings will therefore be $0.05^2 = 0.0025 = 1/400$. The mean length of a double opening will be 0.5 ms (half that for a single opening because shutting of either of the two channels will terminate it), so the mean time between double openings (i.e. the mean length of a run of single openings) will be about 400×0.5 ms = 200 ms. The observed record was open for 10% of the time so the 200 ms run of single openings will contain about 20 single openings on average. If the observed run of single openings is very much longer than this then it is unlikely that there were two active channels present. This argument will next be presented in a rather more precise way.

Runs of single openings

Denote as P_{o2} the probability that a channel is open during an observed run of single openings that originates from two independent channels, i.e. P_{o2} is the fraction of time for which the channel is open during the run. If this value is not too high we can take, as an approximation, the probability that an individual channel is open, P_{o1}, as half of this, so

$$P_{o1} \approx P_{o2}/2. \tag{1}$$

The probability, P_d say, of a double opening, given that the two channels are independent, i.e. the fraction of time occupied by double openings, will be

$$P_d = P_{o1}^2 = \mu_d/(\mu_d + \mu_g),$$

where μ_d represents the mean length of a double opening, and μ_g is the mean length of the interval between double openings. Now $\mu_d = \mu_{o1}/2$ where μ_{o1} is the mean length of the opening for one channel (see below). The lengths of the single openings (μ_{o2} say) seen in a run of single openings will be shorter than μ_{o1} in general, and will not be the same for all single openings, but at low opening rates we can take them to be approximately equal to μ_{o1} (this error partially compensates for that implicit in (1); see below), so we obtain

$$\mu_g/\mu_{o1} \approx \mu_g/\mu_{o2} \approx 0.5\left(\frac{1-P_{o2}^2/4}{P_{o2}^2/4}\right). \tag{2}$$

If the run of single openings contains r openings then the length of time between double openings (see figure 1) can be written, approximately, as

$$\mu_g \approx (r+1)\,\mu_{s2} + (r+2)\,\mu_{o2}, \tag{3}$$

where μ_{s2} is the mean length of the shut time in a run of single openings. Now $P_{o2} \approx \mu_{o2}/(\mu_{o2}+\mu_{s2})$, so,

$$\mu_{s2}/\mu_{o2} \approx (1-P_{o2})/P_{o2}.$$

The mean length of the run (as a multiple of the length of a single opening), μ_r say, is therefore

$$\mu_r \approx \mu_g/\mu_{o2}-(\mu_{s2}/\mu_{o2}+2), \tag{4}$$

and the mean number of single openings in a run is approximately

$$\mathbb{E}(r) \approx \mu_r/(1+\mu_{s2}/\mu_{o2}) = (2/P_{o2})(1-0.5P_{o2}-0.75P_{o2}^2). \tag{5}$$

Thus, at low enough values for P_{o2}, we have simply $\mathbb{E}(r) \approx 2/P_{o2}$. The relation in (5) is plotted in figure 2, together with approximate upper limits for $P = 0.05$, 0.01 and 0.001 calculated as $3\mathbb{E}(r)$, $4.6\mathbb{E}(r)$ and $6.9\mathbb{E}(r)$, respectively. These limits would be exact for an exponentially distributed variable, for example $-\log(0.01) = 4.6$.

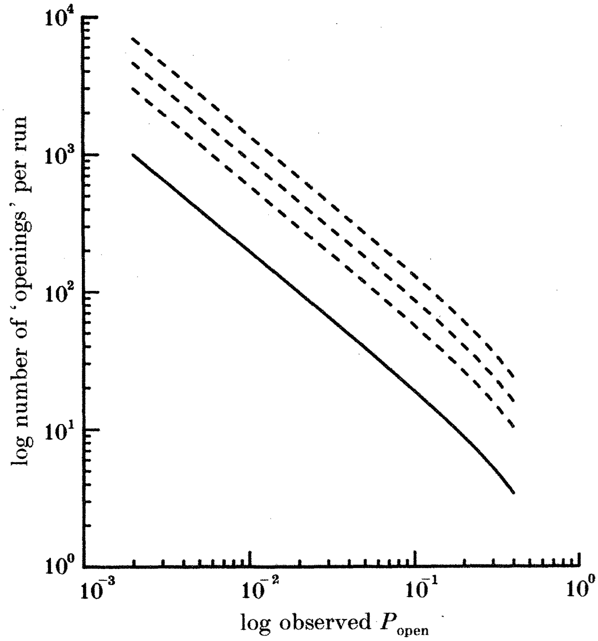

FIGURE 2. The mean number of openings per run of single openings calculated from the approximation given in the text, as a function of P_{o2}. The dashed lines show the approximate upper limits for the number of openings per run for $P = 0.05$, 0.01 and 0.001.

Mean open time within a run

An approximate value can also be obtained for the mean length of an opening within a run, which will be shorter than the mean length of openings for a single channel (because when an opening happens to be long it is more likely that a second channel will open before it shuts, so producing a double opening). From the results of Yeo *et al.* (1989) the distribution of isolated single openings ($f_{Z_{1AB}}$ in their notation) has eigenvalues $(\lambda_i^o+\lambda_j^s)$ where λ_i^o and λ_j^s are the eigenvalues for the distributions of open and shut times for one channel. When there is only one open state (as in the examples below) the distribution of open times within a run is very close to a single exponential with eigenvalue $(\lambda_1^o + \lambda_1^s)$ where $1/\lambda_1^o = \mu_{o1}$ is the mean open time, and $1/\lambda_1^s = \mu_{s1}$ say, is the time constant for the slowest shut-time component, for one channel. The latter is approximately twice the observed value of the slowest shut-time component in the run, so $\mu_{s1} \approx 2\mu_{s2}$. The factor by which an opening in a run is shorter than that for a single channel is thus

$$\mu_{o2}/\mu_{o1} \approx (1+\mu_{o1}/\mu_{s1})^{-1} \approx (1-1.5P_{o2})/(1-P_{o2}), \tag{6}$$

which is detailed in table 1. This correction could be incorporated in (2), and also as an approximate correction in (1) because P_{o1} is greater than $P_{o2}/2$ to an extent that depends largely on the fact that μ_{o2} is less than μ_{o1}. These extra corrections partly cancel each other and give values that are only slightly smaller than those in table 1.

TABLE 1. APPROXIMATE RESULTS FOR RUNS OF SINGLE OPENINGS WITH TWO CHANNELS

P_{o2}	$\mathbb{E}(r)$	$4.6\mathbb{E}(r)$	μ_r	$4.6\mu_r$	μ_{o2}/μ_{o1}
0.01	199.0	915	19.9×10^3	91.5×10^3	0.995
0.025	79.0	363	3.16×10^3	14.5×10^3	0.987
0.05	38.9	179	778.0	3.58×10^3	0.974
0.10	18.8	87	188.0	867.0	0.944
0.20	8.7	40	43.5	200.0	0.875
0.30	5.2	24	17.4	80.0	0.786
0.40	3.4	16	8.5	39.0	0.667

The approximate values for the mean number of openings per run, $\mathbb{E}(r)$, and for the run length (relative to the length of a single opening), are tabulated (table 1) for various values of the observable quantity, P_{o2}. To the extent that the distribution of these values is close to a simple exponential (or geometric) distribution, the value that will be exceeded with 1% probability can be found as 4.6-times the mean. This upper confidence limit is also shown in table 1. These results will be compared with those of the exact calculations, which are described next.

PROPERTIES OF RUNS OF OPENINGS THAT CONTAIN NO DOUBLE OPENINGS

The properties of such runs cannot be derived by the methods of Yeo *et al.* (1989), but can be inferred from the two-channel Q matrix, as in Colquhoun & Hawkes (1977).

For example, when two channels are present, each having one open state and three shut states, then the two channels taken together have one doubly open state, three singly open states, and six shut states (as illustrated in the examples below). In general we define the subset of doubly open states as subset $\mathscr{A}$, the singly open states as subset $\mathscr{B}$, and the shut states as subset $\mathscr{F}$. The notation $\mathscr{F}$ is used for all shut states, rather than $\mathscr{C}$, because the shut states will be subdivided later, when the distribution of bursts is considered. The nature of these states is indicated for the specific mechanisms discussed below.

Notation

The notation used here is the same as that used by Colquhoun & Hawkes (1982). The transition rates between states are in the matrix $\boldsymbol{Q}$. Submatrices of $\boldsymbol{Q}$ that correspond with particular subsets of states are denoted $\boldsymbol{Q}_{\mathscr{AB}}$, etc. $\boldsymbol{G}^*_{\mathscr{AB}}(s)$ denotes $(s\boldsymbol{I}-\boldsymbol{Q}_{\mathscr{AA}})^{-1}\,\boldsymbol{Q}_{\mathscr{AB}}$, where $(s\boldsymbol{I}-\boldsymbol{Q}_{\mathscr{AA}})^{-1}$ is the Laplace transform of $\exp(\boldsymbol{Q}_{\mathscr{AA}}\,t)$. The matrix $\boldsymbol{G}^*_{\mathscr{AB}}(0)$, which is denoted $\boldsymbol{G}_{\mathscr{AB}}$ for brevity, contains transition probabilities from states in $\mathscr{A}$ to states in $\mathscr{B}$ that allow for any number of transitions within $\mathscr{A}$ states before eventual exit to a $\mathscr{B}$ state.

A random variable is denoted, for example, $\tilde{r}$, of which r is a particular value.

The initial vector

A run of single openings is defined to start at the beginning of a single opening, and to end at the end of the last single opening in the run (which is followed by shutting and then a double opening (i.e. by $\mathscr{F}\rightarrow\mathscr{B}\rightarrow\mathscr{A}$), as illustrated in figure 1. There are at least three ways in which the start of a run could be defined.

1. If the run is considered to start after a double opening (i.e. to run from one double to the next) then it is preceded by $\mathscr{A}\rightarrow\mathscr{B}\rightarrow\mathscr{F}$, and then $\mathscr{F}\rightarrow\mathscr{B}$ to start the first single opening of the run. This sort of run is labelled run 2 in figure 1. In this case the relative probabilities of starting the shut period in the various $\mathscr{F}$ states are given by

$$\boldsymbol{\phi}_0 = p_{\mathscr{A}}(\infty)(\boldsymbol{Q}_{\mathscr{AB}}\,\boldsymbol{G}_{\mathscr{BF}}+\boldsymbol{Q}_{\mathscr{AF}})/d, \tag{7}$$

where $p(\infty)$ contains the equilibrium occupancy of each of the individual states, and d is the sum of the elements in the numerator so the probabilities sum to unity. Note, however, that $\boldsymbol{Q}_{\mathscr{AF}}=0$ for the two-channel problem because direct transition from doubly-open to both shut is impossible. The probabilities that the first opening in a run starts in each of the various $\mathscr{B}$ are given by $\boldsymbol{\phi}_0\,\boldsymbol{G}_{\mathscr{FB}}$.

2. In practice an analysis is always started at the first opening, i.e. the channel(s) are initially shut, and the run would be rejected if a double-opening were seen. It is therefore closer to experimental reality to take the relative probabilities of starting in the various $\mathscr{F}$ states as simply $p_{\mathscr{F}}(\infty)$ so we take

$$\boldsymbol{\phi}_0 = p_{\mathscr{F}}(\infty)/p_{\mathscr{F}}(\infty)\,u_{\mathscr{F}}, \tag{8}$$

where $u_{\mathscr{F}}$ is a column vector of ones that sums the terms in the numerator. The condition of the channels at the very start of the recording is usually indeterminate because of artefacts that accompany formation of the patch. It is possible that the recording could start in the middle of an opening (in a $\mathscr{B}$ state with relative probabilities $p_{\mathscr{B}}(\infty)$), but, if it did, this opening would not be measured in practice; we would wait until it had shut (probabilities in $\boldsymbol{G}_{\mathscr{BF}}$) and the timing of the run of single openings would start at the beginning of the next single opening. This suggests that the initial vector that most accurately describes experimental practice is

$$\boldsymbol{\phi}_0 = (p_{\mathscr{F}}(\infty)+p_{\mathscr{B}}(\infty)\,\boldsymbol{G}_{\mathscr{BF}})/(p_{\mathscr{F}}(\infty)+p_{\mathscr{B}}(\infty)\,\boldsymbol{G}_{\mathscr{BF}})\,u_{\mathscr{F}}. \tag{9}$$

This definition of a run is labelled run 1 in figure 1.

In each case the relative probabilities of starting in each of the $\mathscr{B}$ states for the first opening of the run is given by $\boldsymbol{\phi}_0\,\boldsymbol{G}_{\mathscr{FB}}$.

The final vector

The description above of the end of a run implies that it is described as

$$e_0 = (\boldsymbol{G}_{\mathscr{F}\mathscr{B}}\boldsymbol{G}_{\mathscr{B}\mathscr{A}} + \boldsymbol{G}_{\mathscr{F}\mathscr{A}})\,u_{\mathscr{A}}, \tag{10}$$

but again note that $\boldsymbol{G}_{\mathscr{F}\mathscr{A}} = 0$ in the case of the two-channel problem.

The distribution of the duration of a run of single openings

There must be at least one single opening to constitute a run. If there are r openings, the first opening is followed by $r-1$ oscillations from $\mathscr{F} \to \mathscr{B}$ and back so, by the method described by Colquhoun & Hawkes (1982), the Laplace transform of the probability density function (PDF) of the duration of the run has the form

$$\boldsymbol{\phi}_0\,\boldsymbol{G}_{\mathscr{F}\mathscr{B}}\,\boldsymbol{G}^*_{\mathscr{B}\mathscr{F}}(s)[\boldsymbol{G}^*_{\mathscr{F}\mathscr{B}}(s)\,\boldsymbol{G}^*_{\mathscr{B}\mathscr{F}}(s)]^{r-1}e_0. \tag{11}$$

When this is summed over all possibilities, $r = 1, 2, \ldots, \infty$, and normalized so the area of the PDF is unity, we obtain

$$f^*(s) = \boldsymbol{\phi}_0\,\boldsymbol{G}_{\mathscr{F}\mathscr{B}}\,\boldsymbol{G}^*_{\mathscr{B}\mathscr{F}}(s)[\boldsymbol{I} - \boldsymbol{G}^*_{\mathscr{F}\mathscr{B}}(s)\,\boldsymbol{G}^*_{\mathscr{B}\mathscr{F}}(s)]^{-1}e_0/\boldsymbol{\phi}_0\,\boldsymbol{H}_{\mathscr{F}\mathscr{F}}\,u_{\mathscr{F}}, \tag{12}$$

where we define

$$\boldsymbol{H}_{\mathscr{F}\mathscr{F}} = \boldsymbol{G}_{\mathscr{F}\mathscr{B}}\,\boldsymbol{G}_{\mathscr{B}\mathscr{F}}\,, \tag{13}$$

and the normalizing constant in the denominator is just the probability that a run contains at least one single opening (see (16) below). The Laplace transform in (12) can be inverted as described by Colquhoun & Hawkes (1982) to obtain the PDF as

$$f(t) = \boldsymbol{\phi}_0\,\boldsymbol{G}_{\mathscr{F}\mathscr{B}}[\exp \boldsymbol{Q}_{\mathscr{E}\mathscr{E}}\,t)]_{\mathscr{B}\mathscr{B}}\,\boldsymbol{Q}_{\mathscr{B}\mathscr{F}}\,e_0/\boldsymbol{\phi}_0\,\boldsymbol{H}_{\mathscr{F}\mathscr{F}}\,u_{\mathscr{F}}. \tag{14}$$

In this result, the subset $\mathscr{E}$ is defined as all shut or singly open states (i.e. $\mathscr{E} = \mathscr{B} \cup \mathscr{F}$); the subscript $\mathscr{B}\mathscr{B}$ indicates the $\mathscr{B}\mathscr{B}$ subsection (i.e. the top left-hand $k_{\mathscr{B}} \times k_{\mathscr{B}}$ elements) of the matrix $\exp(\boldsymbol{Q}_{\mathscr{E}\mathscr{E}}\,t)$. This result is rather like that for the burst length given by Colquhoun & Hawkes (1982), and it can be rationalized in a similar way; the central term represents a sojourn in any of the $\mathscr{E}$ states (the 'run states') that both starts and ends in a $\mathscr{B}$ state.

The distribution of the number of single openings in a run of single openings

This can be found simply by setting $s = 0$ in (11) (see, for example, Colquhoun & Hawkes (1982) to give the probability of r single openings as

$$\boldsymbol{\phi}_0\,\boldsymbol{H}^r_{\mathscr{F}\mathscr{F}}\,e_0 \quad (r \geqslant 0). \tag{15}$$

However, a run as defined above must have at least one single opening and,

$$\sum_{r=1}^{\infty} \boldsymbol{\phi}_0\,\boldsymbol{H}^r_{\mathscr{F}\mathscr{F}}\,e_0 = \boldsymbol{\phi}_0\,\boldsymbol{H}_{\mathscr{F}\mathscr{F}}\,u_{\mathscr{F}}. \tag{16}$$

Note that this result involves the identity $(\boldsymbol{I} - \boldsymbol{H}_{\mathscr{F}\mathscr{F}})^{-1}\,e_0 = u_{\mathscr{F}}$.

The required result is therefore

$$P(r) = \boldsymbol{\phi}_0\,\boldsymbol{H}^r_{\mathscr{F}\mathscr{F}}\,e_0/\boldsymbol{\phi}_0\,\boldsymbol{H}_{\mathscr{F}\mathscr{F}}\,u_{\mathscr{F}}, \quad (r \geqslant 1), \tag{17}$$

and the mean number of single openings per run is

$$\mathbb{E}(\tilde{r}) = \boldsymbol{\phi}_0 \boldsymbol{H}_{\mathscr{F}\mathscr{F}}(\boldsymbol{I}-\boldsymbol{H}_{\mathscr{F}\mathscr{F}})^{-1} u_{\mathscr{F}} / \boldsymbol{\phi}_0 \boldsymbol{H}_{\mathscr{F}\mathscr{F}} u_{\mathscr{F}}. \tag{18}$$

The cumulative form of this distribution is

$$P(\tilde{r} \geqslant k) = \boldsymbol{\phi}_0 \boldsymbol{H}^k_{\mathscr{F}\mathscr{F}} u_{\mathscr{F}} / \boldsymbol{\phi}_0 \boldsymbol{H}_{\mathscr{F}\mathscr{F}} u_{\mathscr{F}}, \quad (k \geqslant 1). \tag{19}$$

The distribution of open times within a run of single openings

The open times within a run consist of all single openings that start with a shut → open transition, and end with an open → shut transition; their distribution is given by Yeo *et al.* (1989) (as $f_{Z_{1AB}}$ in their notation). The same result can be obtained, rather less simply, from the two-channel $\boldsymbol{Q}$ matrix. However, the advantage of the latter approach is that it allows us to look at the distributions of the first, second, etc. opening in a run (and similarly at the first, second, etc. shutting in a run). This is potentially important because these distributions are in fact not all the same. This results from the fact that there are correlations between the lengths of one opening and the next, as might be expected from the fact that there are several routes between the $\mathscr{B}$ states and the $\mathscr{F}$ states for both of the models that are discussed below, though neither of these mechanisms would show any correlations if only one channel were present (see, for example, Fredkin *et al.* (1985); Colquhoun & Hawkes (1987)). However, in all of the numerical calculations given below the differences between the distributions of the first, second, etc. openings in a run were always so small that they would not be detectable experimentally.

The PDF of the length of the kth single opening in a run of r single openings is, by arguments exactly like those used by Colquhoun & Hawkes (1982) for the openings in a burst:

$$f_{k,r}(t) = \boldsymbol{\phi}_0 \boldsymbol{G}_{\mathscr{F}\mathscr{B}} \boldsymbol{H}^{k-1}_{\mathscr{B}\mathscr{B}} \exp(\boldsymbol{Q}_{\mathscr{B}\mathscr{B}} t)(-\boldsymbol{Q}_{\mathscr{B}\mathscr{B}}) \boldsymbol{H}^{r-k}_{\mathscr{B}\mathscr{B}} \boldsymbol{G}_{\mathscr{B}\mathscr{F}} e_0 / \boldsymbol{\phi}_0 \boldsymbol{H}_{\mathscr{F}\mathscr{F}} u_{\mathscr{F}}.P(r), \quad (r \geqslant k), \tag{20}$$

where we define

$$\boldsymbol{H}_{\mathscr{B}\mathscr{B}} = \boldsymbol{G}_{\mathscr{B}\mathscr{F}} \boldsymbol{G}_{\mathscr{F}\mathscr{B}},$$

and $P(r)$, $(r \geqslant 1)$ is given by (17). To obtain the distribution of the kth single opening, regardless of r, we multiply by $P(r)$ and sum over $r \geqslant k$, to give, when properly normalized to unit area:

$$f_k(t) = \boldsymbol{\phi}_0 \boldsymbol{G}_{\mathscr{F}\mathscr{B}} \boldsymbol{H}^{k-1}_{\mathscr{B}\mathscr{B}} \exp(\boldsymbol{Q}_{\mathscr{B}\mathscr{B}} t)(-\boldsymbol{Q}_{\mathscr{B}\mathscr{B}}) \boldsymbol{G}_{\mathscr{B}\mathscr{F}} u_{\mathscr{F}} / \boldsymbol{\phi}_0 \boldsymbol{H}_{\mathscr{F}\mathscr{F}} u_{\mathscr{F}}.P(\tilde{r} \geqslant k), \quad (k \geqslant 1), \tag{21}$$

with mean

$$\mu = \boldsymbol{\phi}_0 \boldsymbol{G}_{\mathscr{F}\mathscr{B}} \boldsymbol{H}^{k-1}_{\mathscr{B}\mathscr{B}} (-\boldsymbol{Q}^{-1}_{\mathscr{B}\mathscr{B}}) \boldsymbol{G}_{\mathscr{B}\mathscr{F}} u_{\mathscr{F}} / \boldsymbol{\phi}_0 \boldsymbol{H}_{\mathscr{F}\mathscr{F}} u_{\mathscr{F}}.P(\tilde{r} \geqslant k). \tag{22}$$

The denominator, as might be expected, involves the probability that a run contains at least k openings. The unconditional distribution of all open times in the run is found by multiplying this by $P(\tilde{r} \geqslant k)$ and summing over $k = 1, 2, \ldots, \infty$. When normalized, this gives

$$f(t) = \boldsymbol{\phi}_0 \boldsymbol{G}_{\mathscr{F}\mathscr{B}}(\boldsymbol{I}-\boldsymbol{H}_{\mathscr{B}\mathscr{B}})^{-1} \exp(\boldsymbol{Q}_{\mathscr{B}\mathscr{B}} t)(-\boldsymbol{Q}_{\mathscr{B}\mathscr{B}}) \boldsymbol{G}_{\mathscr{B}\mathscr{F}} u_{\mathscr{F}} / \boldsymbol{\phi}_0 \boldsymbol{H}_{\mathscr{F}\mathscr{F}} u_{\mathscr{F}}.\mathbb{E}(\tilde{r}). \tag{23}$$

The overall mean open time, μ_{o2} say, is therefore

$$\mu_{o2} = \boldsymbol{\phi}_0 \boldsymbol{G}_{\mathscr{F}\mathscr{B}}(\boldsymbol{I}-\boldsymbol{H}_{\mathscr{B}\mathscr{B}})^{-1}(-\boldsymbol{Q}_{\mathscr{B}\mathscr{B}})^{-1} \boldsymbol{G}_{\mathscr{B}\mathscr{F}} u_{\mathscr{F}} / \boldsymbol{\phi}_0 \boldsymbol{H}_{\mathscr{F}\mathscr{F}} u_{\mathscr{F}}.\mathbb{E}(\tilde{r}). \tag{24}$$

The distribution of shut times within a run of single openings

Exactly similar arguments give, for the kth shut period in a run with r single openings:

$$f_{k,r}(t) = \boldsymbol{\phi}_0 \boldsymbol{G}_{\mathscr{F}\mathscr{B}} \boldsymbol{H}_{\mathscr{B}\mathscr{B}}^{k-1} \boldsymbol{G}_{\mathscr{B}\mathscr{F}} \exp(\boldsymbol{Q}_{\mathscr{F}\mathscr{F}}\, t)\, \boldsymbol{Q}_{\mathscr{F}\mathscr{B}} \boldsymbol{H}_{\mathscr{B}\mathscr{B}}^{r-k-1} \boldsymbol{G}_{\mathscr{B}\mathscr{F}}\, e_0 / \boldsymbol{\phi}_0 \boldsymbol{H}_{\mathscr{F}\mathscr{F}}\, u_{\mathscr{F}}.P(r)$$
$$= \boldsymbol{\phi}_0 \boldsymbol{H}_{\mathscr{F}\mathscr{F}}^{k} \exp(\boldsymbol{Q}_{\mathscr{F}\mathscr{F}}\, t)(-\boldsymbol{Q}_{\mathscr{F}\mathscr{F}})\, \boldsymbol{H}_{\mathscr{F}\mathscr{F}}^{r-k}\, e_0 / \boldsymbol{\phi}_0 \boldsymbol{H}_{\mathscr{F}\mathscr{F}} u_{\mathscr{F}}.P(r), \quad k = 1, \ldots, r-1. \tag{25}$$

For the kth shut period regardless of r we get

$$f_k(t) = \boldsymbol{\phi}_0 \boldsymbol{H}_{\mathscr{F}\mathscr{F}}^{k} \exp(\boldsymbol{Q}_{\mathscr{F}\mathscr{F}}\, t)(-\boldsymbol{Q}_{\mathscr{F}\mathscr{F}})\, \boldsymbol{H}_{\mathscr{F}\mathscr{F}}\, u_{\mathscr{F}} / \boldsymbol{\phi}_0 \boldsymbol{H}_{\mathscr{F}\mathscr{F}}\, u_{\mathscr{F}}.P(\tilde{r} \geqslant k+1)$$
$$k = 1, \ldots, r-1, \tag{26}$$

where the denominator involves the probability that a run contains at least $k+1$ openings, i.e. that it contains at least k shut periods. The overall distribution of shut times within a run is

$$f(t) = \boldsymbol{\phi}_0 \boldsymbol{H}_{\mathscr{F}\mathscr{F}}(I - \boldsymbol{H}_{\mathscr{F}\mathscr{F}})^{-1} \exp(\boldsymbol{Q}_{\mathscr{F}\mathscr{F}}\, t)(-\boldsymbol{Q}_{\mathscr{F}\mathscr{F}})\, \boldsymbol{H}_{\mathscr{F}\mathscr{F}}\, u_{\mathscr{F}} / \boldsymbol{\phi}_0 \boldsymbol{H}_{\mathscr{F}\mathscr{F}}\, u_{\mathscr{F}}.[\mathbb{E}(\tilde{r}) - 1]. \tag{27}$$

This will not be exactly the same as the distribution of all shut times given by Yeo *et al.* (1989) because it excludes the shut times that precede the first opening in the run, and that following the last opening in the run. The overall mean shut time, μ_{s2} say, for shuttings within a run is given by

$$\mu_{s2} = \boldsymbol{\phi}_0 \boldsymbol{H}_{\mathscr{F}\mathscr{F}}(I - \boldsymbol{H}_{\mathscr{F}\mathscr{F}})^{-1}(-\boldsymbol{Q}_{\mathscr{F}\mathscr{F}}^{-1})\, \boldsymbol{H}_{\mathscr{F}\mathscr{F}}\, u_{\mathscr{F}} / \boldsymbol{\phi}_0 \boldsymbol{H}_{\mathscr{F}\mathscr{F}}\, u_{\mathscr{F}}.[\mathbb{E}(\tilde{r}) - 1]. \tag{28}$$

The probability of being open during a run of single openings

This was calculated as the mean total open time in a run divided by the mean length of the run, i.e. from (18), (24) and (28), as

$$P_{o2} = \frac{\mathbb{E}(\tilde{r})\, \mu_{o2}}{\mathbb{E}(\tilde{r})\, \mu_{o2} + [\mathbb{E}(\tilde{r}) - 1]\, \mu_{s2}}. \tag{29}$$

Properties of runs of bursts that contain no double openings

It will be seen in the numerical examples given below, that to test adequately the approximate relations given at the beginning of the paper, it is necessary to consider the possibility that openings may occur in *bursts* of closely spaced openings separated from each other by brief shut periods. This is necessary because, if two such bursts overlap they will almost certainly produce one or more double openings. Bursts are commonly observed in practice, and can be treated theoretically along the lines described by Colquhoun & Hawkes (1982).

A *burst* of openings, for a single channel, will be defined here as a series of openings, the openings being separated by sojourns in shut states that all have at least one agonist molecular bound (and possibly a blocker molecule too). This definition of a burst corresponds to a single *activation* of the channel by agonist. The extent to which such bursts are distinguishable from one another on the experimental record depends, of course, on the mechanism postulated, on the particular values for the rate constants, and, especially, on the concentration of agonist that controls the interval between individual channel activations. The question of experimental definition of bursts will be considered again later.

When two channels are active we shall define a burst in the observed record as a period during which either of the individual channels is in a burst, or both are (i.e. bursts in the two individual channels overlap). We are concerned here with runs of single bursts (i.e. a consecutive series of bursts that contains no double openings), as illustrated in figure 3 (see below for details). If the channel is open for most of the time during a burst (i.e. the shut times within the burst are brief relative to the openings) then overlapping of bursts will almost certainly result in double openings, so ending the run. On the other hand, bursts that contain long gaps could well overlap without producing double openings (see numerical results).

This definition of bursts requires that subsets of states should now be defined as follows. The definitions of subsets $\mathscr{A}$ and $\mathscr{B}$ is as before, but the shut states, $\mathscr{F}$, are now subdivided.

Subset $\mathscr{A}$ contains the $k_{\mathscr{A}}$ doubly open state(s).

Subset $\mathscr{B}$ contains the $k_{\mathscr{B}}$ singly open states.

Subset $\mathscr{C}$ contains all $k_{\mathscr{C}}$ states for which both channels are shut, but one or both channels has at least one agonist molecule bound.

Subset $\mathscr{D}$ contains $k_{\mathscr{D}}$ shut state(s) for which neither channel has an agonist molecule bound (so there is only one $\mathscr{D}$ state for both of the particular mechanisms discussed below).

Subset $\mathscr{F} = \mathscr{C} \cup \mathscr{D}$ thus contains all states ($k_{\mathscr{F}} = k_{\mathscr{C}} + k_{\mathscr{D}}$ in number) for which both channels are shut, as before.

We further define, for use later:

Subset $\mathscr{E} = \mathscr{B} \cup \mathscr{C}$, the 'burst states', $k_{\mathscr{E}} = k_{\mathscr{B}} + k_{\mathscr{C}}$ in number, and Subset $\mathscr{G} = \mathscr{B} \cup \mathscr{C} \cup \mathscr{D}$, the 'run of single burst states'.

A run of bursts as defined here bears some analogy to the clusters of bursts analysed by Colquhoun & Hawkes (1982), so the subsets defined above are similar to those used for analysis of clusters, and the results derive below often bear a resemblance to those derived for clusters.

For the particular case of two independent channels, with which we are concerned here, there can be no direct transitions from, for example, $\mathscr{A}$ states to $\mathscr{C}$ states, so

$$\boldsymbol{Q}_{\mathscr{A}\mathscr{C}} = \boldsymbol{Q}_{\mathscr{C}\mathscr{A}} = \boldsymbol{O}; \quad \boldsymbol{Q}_{\mathscr{A}\mathscr{F}} = \boldsymbol{Q}_{\mathscr{F}\mathscr{A}} = \boldsymbol{O}; \quad \boldsymbol{Q}_{\mathscr{B}\mathscr{D}} = \boldsymbol{Q}_{\mathscr{D}\mathscr{B}} = \boldsymbol{O}, \text{ etc.} \tag{30}$$

The initial vector

A run of bursts can be defined in ways that are analogous with those used to define runs of openings. The method used to derive (9) corresponds most precisely to experimental practice, so its analogue will be used for bursts. If the observed record starts in the middle of a burst, this burst, and the subsequent shut period, are ignored and the run is defined as starting at the beginning of the next burst, as illustrated in figure 3.

The initial vector for a run of bursts, analogous to (9), is thus

$$\boldsymbol{\phi}_{\mathrm{b}} = (\boldsymbol{p}_{\mathscr{D}}(\infty) + \boldsymbol{p}_{\mathscr{E}}(\infty)\,\boldsymbol{G}_{\mathscr{E}\mathscr{D}})/(\boldsymbol{p}_{\mathscr{D}}(\infty) + \boldsymbol{p}_{\mathscr{E}}(\infty)\,\boldsymbol{G}_{\mathscr{E}\mathscr{D}})\,\boldsymbol{u}_{\mathscr{D}}. \tag{31}$$

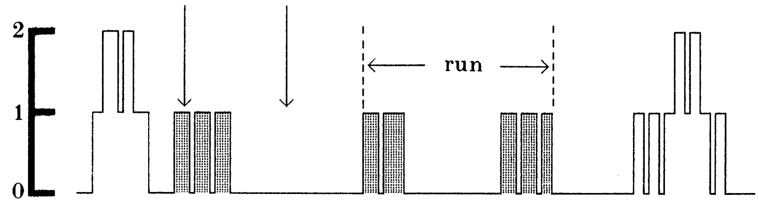

FIGURE 3. Definition of a run of single bursts. The scale on the left indicates the number of channels that are open. Individual bursts are shaded. For runs that started at either of the points marked by vertical arrows, the initial vector used for the calculations defines the run of single bursts as the section of the record labelled run. In this example, the run contains two bursts.

This gives the relative probabilities of being in the various $\mathscr{D}$ states before the first burst of a run. When there is only one $\mathscr{D}$ state, as in the examples below, $\boldsymbol{\phi}_\mathrm{b}$ will be a scalar, equal to unity. The relative probabilities of starting the first burst of the run in each of the $\mathscr{B}$ states will thus be

$$\boldsymbol{\phi}_\mathrm{b}[\boldsymbol{G}_{\mathscr{F}\mathscr{B}}]_{\mathscr{D}\mathscr{B}}, \tag{32}$$

where the $\mathscr{D}\mathscr{B}$ subsection of $\boldsymbol{G}_{\mathscr{F}\mathscr{B}}$ can be expanded (see, for example, Colquhoun & Hawkes (1982) appendix 1) in the form

$$[\boldsymbol{G}_{\mathscr{F}\mathscr{B}}]_{\mathscr{D}\mathscr{B}} = (\boldsymbol{I}-\boldsymbol{G}_{\mathscr{D}\mathscr{C}}\,\boldsymbol{G}_{\mathscr{C}\mathscr{D}})^{-1}\boldsymbol{G}_{\mathscr{D}\mathscr{C}}\,\boldsymbol{G}_{\mathscr{C}\mathscr{B}} \tag{33}$$

This describes pathways from $\mathscr{D}$ to $\mathscr{B}$ that start in a $\mathscr{D}$ state, then oscillate any number of times between shut states ($\mathscr{F} = \mathscr{C} \cup \mathscr{D}$) before leaving via $\mathscr{D} \to \mathscr{C} \to \mathscr{B}$ to start the burst.

The final vector

The end of a run is defined as being at the end of the last burst preceding a burst that contains a double opening, as shown in figure 3. The pathways for the ending of a run of single bursts are described, as there can be no direct path from $\mathscr{F}$ to $\mathscr{A}$, by the end vector

$$e_\mathrm{b} = [\boldsymbol{G}_{\mathscr{F}\mathscr{B}}]_{\mathscr{D}\mathscr{B}} \cdot [\boldsymbol{G}_{\mathscr{E}\mathscr{A}}]_{\mathscr{B}\mathscr{A}} \cdot u_{\mathscr{A}}, \tag{34}$$

where $[\boldsymbol{G}_{\mathscr{F}\mathscr{B}}]_{\mathscr{D}\mathscr{B}}$ was given in (33), and we can, as there can be no direct pathway from $\mathscr{A}$ to $\mathscr{C}$, expand $[\boldsymbol{G}_{\mathscr{E}\mathscr{A}}]_{\mathscr{B}\mathscr{A}}$ as

$$[\boldsymbol{G}_{\mathscr{E}\mathscr{A}}]_{\mathscr{B}\mathscr{A}} = (\boldsymbol{I}-\boldsymbol{G}_{\mathscr{B}\mathscr{C}}\,\boldsymbol{G}_{\mathscr{C}\mathscr{B}})^{-1}\boldsymbol{G}_{\mathscr{B}\mathscr{A}}. \tag{35}$$

This describes paths from the singly open burst ($\mathscr{E}$ states) that starts in $\mathscr{B}$ and oscillates between $\mathscr{B}$ and $\mathscr{C}$ before leaving for a doubly open $\mathscr{A}$ state.

The distribution of the duration of a run of single bursts

This can be derived in a similar way to that used for runs of single openings in (11)–(14) above. The result will, however, be slightly different because the start and end of the run are defined slightly differently (see figures 1 and 3), and because the run of bursts must contain at least one burst (rather than at least one opening). Enumeration of possible routes through a run gives the Laplace transform of the PDF as

$$f^*(s) = \boldsymbol{\phi}_\mathrm{b}[\boldsymbol{G}_{\mathscr{F}\mathscr{B}}]_{\mathscr{D}\mathscr{B}}[\boldsymbol{I}-\boldsymbol{Z}^*_{\mathscr{B}\mathscr{B}}(s)]^{-1}[\boldsymbol{I}-\boldsymbol{G}^*_{\mathscr{B}\mathscr{C}}(s)\,\boldsymbol{G}^*_{\mathscr{C}\mathscr{B}}(s)]^{-1}\boldsymbol{G}^*_{\mathscr{B}\mathscr{C}}(s)\,\boldsymbol{G}_{\mathscr{C}\mathscr{D}}\,e_\mathrm{b}/d, \tag{36}$$

where we define

$$Z^*_{\mathscr{B}\mathscr{B}}(s) = [I - G^*_{\mathscr{B}\mathscr{C}}(s)\, G^*_{\mathscr{C}\mathscr{B}}(s)]^{-1} G^*_{\mathscr{B}\mathscr{C}}(s)\, G^*_{\mathscr{C}\mathscr{D}}(s)\, [I - G^*_{\mathscr{D}\mathscr{C}}(s)\, G^*_{\mathscr{C}\mathscr{D}}(s)]^{-1} G^*_{\mathscr{D}\mathscr{C}}(s)\, G^*_{\mathscr{C}\mathscr{B}}(s), \tag{37}$$

and the denominator, d, is chosen to make the area of the PDF, $f^*(0)$, equal to unity. Note that $Z^*_{\mathscr{B}\mathscr{B}}(s)$ describes the duration of time from the start of one burst to the start of the next. This result, which resembles that for the length of a cluster of bursts in Colquhoun & Hawkes (1982), can be inverted by similar methods to give the PDF as

$$f(t) = \phi_{\mathrm{b}}[G_{\mathscr{F}\mathscr{B}}]_{\mathscr{D}\mathscr{B}}[\exp(Q_{\mathscr{G}\mathscr{G}}\, t)]_{\mathscr{B}\mathscr{B}}\, Q_{\mathscr{B}\mathscr{C}}\, G_{\mathscr{C}\mathscr{D}}\, e_{\mathrm{b}} / \phi_{\mathrm{b}}\, Z_{\mathscr{D}\mathscr{D}}\, u_{\mathscr{D}}, \tag{38}$$

which can be expressed as the sum of $k_{\mathscr{G}}$ exponentials. After the start of the first burst in $\mathscr{B}$, the exponential term represents a sojourn in the 'run states' ($\mathscr{G} = \mathscr{B} \cup \mathscr{C} \cup \mathscr{D}$) that starts and ends in a singly open ($\mathscr{B}$) state. The mean duration of a run, μ_{r1} say, is

$$\mu_{\mathrm{r1}} = \phi_{\mathrm{b}}[G_{\mathscr{F}\mathscr{B}}]_{\mathscr{D}\mathscr{B}}(I - G_{\mathscr{B}\mathscr{F}}\, G_{\mathscr{F}\mathscr{B}})^{-1}(-Q^{-1}_{\mathscr{B}\mathscr{B}} - G_{\mathscr{B}\mathscr{F}}\, Q^{-1}_{\mathscr{F}\mathscr{F}}\, G_{\mathscr{F}\mathscr{B}})[G_{\mathscr{E}\mathscr{D}}]_{\mathscr{B}\mathscr{D}}\, u_{\mathscr{D}} / \phi_{\mathrm{b}}\, Z_{\mathscr{D}\mathscr{D}}\, u_{\mathscr{D}}. \tag{39}$$

The denominator is a normalizing constant necessitated by the requirement that a run contains at least one burst; it involves $Z_{\mathscr{D}\mathscr{D}}$, which is defined as

$$Z_{\mathscr{D}\mathscr{D}} = [G_{\mathscr{F}\mathscr{B}}]_{\mathscr{D}\mathscr{B}}[G_{\mathscr{E}\mathscr{D}}]_{\mathscr{B}\mathscr{D}}. \tag{40}$$

The expansion of $[G_{\mathscr{F}\mathscr{B}}]_{\mathscr{D}\mathscr{B}}$ was given above in (33), and $[G_{\mathscr{E}\mathscr{D}}]_{\mathscr{B}\mathscr{D}}$ can be similarly expanded, as $G_{\mathscr{B}\mathscr{D}} = 0$, as

$$[G_{\mathscr{E}\mathscr{D}}]_{\mathscr{B}\mathscr{D}} = (I - G_{\mathscr{B}\mathscr{C}}\, G_{\mathscr{C}\mathscr{B}})^{-1} G_{\mathscr{B}\mathscr{C}}\, G_{\mathscr{C}\mathscr{D}}. \tag{41}$$

Thus we can write:

$$Z_{\mathscr{D}\mathscr{D}} = (I - G_{\mathscr{D}\mathscr{C}}\, G_{\mathscr{C}\mathscr{D}})^{-1} G_{\mathscr{D}\mathscr{C}}\, G_{\mathscr{C}\mathscr{B}}(I - G_{\mathscr{B}\mathscr{C}}\, G_{\mathscr{C}\mathscr{B}})^{-1} G_{\mathscr{B}\mathscr{C}}\, G_{\mathscr{C}\mathscr{D}}, \tag{42}$$

which represents oscillations in the shut states that start in $\mathscr{D}$ and end via $\mathscr{D} \to \mathscr{C}$ to a singly open ($\mathscr{B}$) state, followed by oscillations in the burst states ($\mathscr{E} = \mathscr{B} \cup \mathscr{C}$) that start and end in $\mathscr{B}$ and return via $\mathscr{C}$ to $\mathscr{D}$. This expression is thus similar to $Z_{\mathscr{A}\mathscr{A}}$ in Colquhoun & Hawkes (1982), and a notation analogous to theirs would result if we defined $[G_{\mathscr{F}\mathscr{B}}]_{\mathscr{D}\mathscr{B}} \equiv G_{\mathscr{D}(\mathscr{C})\mathscr{B}}$ and $[G_{\mathscr{E}\mathscr{D}}]_{\mathscr{B}\mathscr{D}} \equiv G_{\mathscr{B}(\mathscr{C})\mathscr{D}}$. The matrix $Z_{\mathscr{D}\mathscr{D}}$ represents routes via which a run may continue, so it is intuitively reasonable that it is related to the vector that describes the end of a run by

$$e_{\mathrm{b}} = (I - Z_{\mathscr{D}\mathscr{D}})\, u_{\mathscr{D}}. \tag{43}$$

The derivations of these results use the relations

$$\begin{aligned}[(sI - Q_{\mathscr{G}\mathscr{G}})^{-1}]_{\mathscr{B}\mathscr{B}} &= [I - G^*_{\mathscr{B}\mathscr{F}}(s)\, G^*_{\mathscr{F}\mathscr{B}}(s)]^{-1}(sI - Q_{\mathscr{B}\mathscr{B}})^{-1} \\ &= [I - Z^*_{\mathscr{B}\mathscr{B}}(s)]^{-1}[I - G^*_{\mathscr{B}\mathscr{C}}(s)\, G^*_{\mathscr{C}\mathscr{B}}(s)]^{-1}(sI - Q_{\mathscr{B}\mathscr{B}})^{-1}.\end{aligned} \tag{44}$$

We shall also need later the results found from (44) with $s = 0$,

$$(I - G_{\mathscr{B}\mathscr{F}}\, G_{\mathscr{F}\mathscr{B}})^{-1} = (I - Z_{\mathscr{B}\mathscr{B}})^{-1}(I - G_{\mathscr{B}\mathscr{C}}\, G_{\mathscr{C}\mathscr{B}})^{-1}. \tag{45}$$

The distribution of the number of bursts in a run of single bursts

The derivation follows the same lines as for runs of openings, as in (15)–(19) above. The probability of observing n bursts in a run is

$$\begin{aligned} P_{\mathrm{b}}(n) &= \boldsymbol{\phi}_{\mathrm{b}}\, \boldsymbol{Z}^{n}_{\mathscr{D}\mathscr{D}}\, e_{\mathrm{b}}/\boldsymbol{\phi}_{\mathrm{b}}\, \boldsymbol{Z}_{\mathscr{D}\mathscr{D}}\, u_{\mathscr{D}} \\ &= \boldsymbol{\phi}_{\mathrm{b}}\, \boldsymbol{Z}^{n}_{\mathscr{D}\mathscr{D}}(\boldsymbol{I}-\boldsymbol{Z}_{\mathscr{D}\mathscr{D}})\, u_{\mathscr{D}}/\boldsymbol{\phi}_{\mathrm{b}}\, \boldsymbol{Z}_{\mathscr{D}\mathscr{D}}\, u_{\mathscr{D}}, \quad (n \geqslant 1). \end{aligned} \tag{46}$$

The latter result is the matrix analogue of a geometric distribution, and can be expressed as the sum of $k_{\mathscr{D}}$ geometric distributions. In the examples below, for which $k_{\mathscr{D}} = 1$, it is a simple geometric distribution. The mean number of bursts per run is

$$\mathbb{E}_{\mathrm{b}}(\tilde{n}) = \boldsymbol{\phi}_{\mathrm{b}}\, \boldsymbol{Z}_{\mathscr{D}\mathscr{D}}(\boldsymbol{I}-\boldsymbol{Z}_{\mathscr{D}\mathscr{D}})^{-1} u_{\mathscr{D}}/\boldsymbol{\phi}_{\mathrm{b}}\, \boldsymbol{Z}_{\mathscr{D}\mathscr{D}}\, u_{\mathscr{D}}. \tag{47}$$

The cumulative form of the distribution gives the probability of observing k or more bursts per run as

$$P_{\mathrm{b}}(\tilde{n} \geqslant k) = \boldsymbol{\phi}_{\mathrm{b}}\, \boldsymbol{Z}^{k}_{\mathscr{D}\mathscr{D}}\, u_{\mathscr{D}}/\boldsymbol{\phi}_{\mathrm{b}}\, \boldsymbol{Z}_{\mathscr{D}\mathscr{D}}\, u_{\mathscr{D}}. \tag{48}$$

The distribution of the total time spent in bursts in a run of single bursts

The Laplace transform of this distribution is

$$\begin{aligned} f^{*}(s) &= \boldsymbol{\phi}_{\mathrm{b}} \sum_{r=1}^{\infty} [\boldsymbol{Z}^{*}_{\mathscr{D}\mathscr{D}}(s)]^{r} e_{\mathrm{b}}/\boldsymbol{\phi}_{\mathrm{b}}\, \boldsymbol{Z}_{\mathscr{D}\mathscr{D}}\, u_{\mathscr{D}} \\ &= \boldsymbol{\phi}_{\mathrm{b}}[\boldsymbol{I}-\boldsymbol{Z}^{*}_{\mathscr{D}\mathscr{D}}(s)]^{-1}\boldsymbol{Z}^{*}_{\mathscr{D}\mathscr{D}}(s)\, e_{\mathrm{b}}/\boldsymbol{\phi}_{\mathrm{b}}\, \boldsymbol{Z}_{\mathscr{D}\mathscr{D}}\, u_{\mathscr{D}}, \end{aligned} \tag{49}$$

where we define

$$\boldsymbol{Z}^{*}_{\mathscr{D}\mathscr{D}}(s) = [\boldsymbol{G}_{\mathscr{F}\mathscr{B}}]_{\mathscr{D}\mathscr{B}}[\boldsymbol{I}-\boldsymbol{G}^{*}_{\mathscr{B}\mathscr{C}}(s)\,\boldsymbol{G}^{*}_{\mathscr{C}\mathscr{B}}(s)]^{-1}\boldsymbol{G}^{*}_{\mathscr{B}\mathscr{C}}(s)\,\boldsymbol{G}_{\mathscr{C}\mathscr{D}}. \tag{50}$$

This definition is not exactly analogous with that of $\boldsymbol{Z}^{*}_{\mathscr{B}\mathscr{B}}(s)$ in (37) because s is set to zero here in those terms for which the time is irrelevant. The mean total burst time per run, μ_{tb} say, is thus

$$\mu_{\mathrm{tb}} = \boldsymbol{\phi}_{\mathrm{b}}(\boldsymbol{I}-\boldsymbol{Z}_{\mathscr{D}\mathscr{D}})^{-1}\boldsymbol{M}_{\mathscr{D}\mathscr{D}}\, u_{\mathscr{D}}/\boldsymbol{\phi}_{\mathrm{b}}\, \boldsymbol{Z}_{\mathscr{D}\mathscr{D}}\, u_{\mathscr{D}}, \tag{51}$$

where

$$\begin{aligned} \boldsymbol{M}_{\mathscr{D}\mathscr{D}} &= -\left.\frac{\mathrm{d}\boldsymbol{Z}^{*}_{\mathscr{D}\mathscr{D}}(s)}{\mathrm{d}s}\right|_{s=0} \\ &= -[\boldsymbol{G}_{\mathscr{F}\mathscr{B}}]_{\mathscr{D}\mathscr{B}}(\boldsymbol{I}-\boldsymbol{G}_{\mathscr{B}\mathscr{C}}\,\boldsymbol{G}_{\mathscr{C}\mathscr{B}})^{-1}(\boldsymbol{Q}^{-1}_{\mathscr{B}\mathscr{B}}+\boldsymbol{G}_{\mathscr{B}\mathscr{C}}\,\boldsymbol{Q}^{-1}_{\mathscr{C}\mathscr{C}}\,\boldsymbol{G}_{\mathscr{C}\mathscr{B}})[\boldsymbol{G}_{\mathscr{E}\mathscr{D}}]_{\mathscr{B}\mathscr{D}}. \end{aligned} \tag{52}$$

Distribution of burst lengths in a run of single bursts

For the mth burst in a run with n bursts, the Laplace transform of the PDF will be:

$$f^{*}_{m,n}(s) = \boldsymbol{\phi}_{\mathrm{b}}\, \boldsymbol{Z}^{m-1}_{\mathscr{D}\mathscr{D}}\boldsymbol{Z}^{*}_{\mathscr{D}\mathscr{D}}(s)\, \boldsymbol{Z}^{n-m}_{\mathscr{D}\mathscr{D}}\, e_{\mathrm{b}}/\boldsymbol{\phi}_{\mathrm{b}}\, \boldsymbol{Z}_{\mathscr{D}\mathscr{D}}\, u_{\mathscr{D}}.P_{\mathrm{b}}(n). \tag{53}$$

The overall distribution of the duration of bursts in a run, found by summing over m and n, and normalizing, gives

$$f^{*}(s) = \boldsymbol{\phi}_{\mathrm{b}}(\boldsymbol{I}-\boldsymbol{Z}_{\mathscr{D}\mathscr{D}})^{-1}\boldsymbol{Z}^{*}_{\mathscr{D}\mathscr{D}}(s)(\boldsymbol{I}-\boldsymbol{Z}_{\mathscr{D}\mathscr{D}})^{-1}e_{\mathrm{b}}/\boldsymbol{\phi}_{\mathrm{b}}\, \boldsymbol{Z}_{\mathscr{D}\mathscr{D}}\, u_{\mathscr{D}}.\mathbb{E}_{\mathrm{b}}(\tilde{n}). \tag{54}$$

Inversion gives the PDF as

$$f(t) = \boldsymbol{\phi}_{\mathrm{b}}(\boldsymbol{I}-\boldsymbol{Z}_{\mathscr{D}\mathscr{D}})^{-1}[\boldsymbol{G}_{\mathscr{F}\mathscr{B}}]_{\mathscr{D}\mathscr{B}}[\exp(\boldsymbol{Q}_{\mathscr{E}\mathscr{E}}\, t)]_{\mathscr{B}\mathscr{B}}\, \boldsymbol{Q}_{\mathscr{B}\mathscr{C}}\, \boldsymbol{G}_{\mathscr{C}\mathscr{D}}\, u_{\mathscr{D}}/\boldsymbol{\phi}_{\mathrm{b}}\, \boldsymbol{Z}_{\mathscr{D}\mathscr{D}}\, u_{\mathscr{D}}.\mathbb{E}_{\mathrm{b}}(\tilde{n}), \tag{55}$$

with mean
$$\mu = \boldsymbol{\phi}_{\mathrm{b}}(\boldsymbol{I}-\boldsymbol{Z}_{\mathscr{D}\mathscr{D}})^{-1}\boldsymbol{M}_{\mathscr{D}\mathscr{D}}\,u_{\mathscr{D}}/\boldsymbol{\phi}_{\mathrm{b}}\,\boldsymbol{Z}_{\mathscr{D}\mathscr{D}}\,u_{\mathscr{D}}.\mathbb{E}_{\mathrm{b}}(\tilde{n}), \tag{56}$$
which is, as expected, shorter than the mean burst time per run by a factor $\mathbb{E}_{\mathrm{b}}(\tilde{n})$.

The distribution of the number of openings per burst in a run of single bursts

The probability of observing r openings in the mth burst of a run that contains n bursts is

$$P(r;m,n) = \boldsymbol{\phi}_{\mathrm{b}}\,\boldsymbol{Z}_{\mathscr{D}\mathscr{D}}^{m-1}[\boldsymbol{G}_{\mathscr{F}\mathscr{B}}]_{\mathscr{D}\mathscr{B}}(\boldsymbol{G}_{\mathscr{B}\mathscr{C}}\,\boldsymbol{G}_{\mathscr{C}\mathscr{B}})^{r-1}\boldsymbol{G}_{\mathscr{B}\mathscr{C}}\,\boldsymbol{G}_{\mathscr{C}\mathscr{D}}\,\boldsymbol{Z}_{\mathscr{D}\mathscr{D}}^{n-m}e_{\mathrm{b}}/\boldsymbol{\phi}_{\mathrm{b}}\,\boldsymbol{Z}_{\mathscr{D}\mathscr{D}}\,u_{\mathscr{D}}.P_{\mathrm{b}}(n), \quad (r \geqslant 1;\ m \leqslant n). \tag{57}$$

The probability of r openings in the mth burst, regardless of n, is found by multiplying this by $P_{\mathrm{b}}(n)$, summing over $n = m, \ldots, \infty$, and normalizing to give

$$\begin{aligned} P(r;m) &= \boldsymbol{\phi}_{\mathrm{b}}\,\boldsymbol{Z}_{\mathscr{D}\mathscr{D}}^{m-1}[\boldsymbol{G}_{\mathscr{F}\mathscr{B}}]_{\mathscr{D}\mathscr{B}}(\boldsymbol{G}_{\mathscr{B}\mathscr{C}}\,\boldsymbol{G}_{\mathscr{C}\mathscr{B}})^{r-1}\boldsymbol{G}_{\mathscr{B}\mathscr{C}}\,\boldsymbol{G}_{\mathscr{C}\mathscr{D}}(\boldsymbol{I}-\boldsymbol{Z}_{\mathscr{D}\mathscr{D}})^{-1}e_{\mathrm{b}}/\boldsymbol{\phi}_{\mathrm{b}}\,\boldsymbol{Z}_{\mathscr{D}\mathscr{D}}^{m}u_{\mathscr{D}} \\ &= \boldsymbol{\phi}_{\mathrm{b}}\,\boldsymbol{Z}_{\mathscr{D}\mathscr{D}}^{m-1}[\boldsymbol{G}_{\mathscr{F}\mathscr{B}}]_{\mathscr{D}\mathscr{B}}(\boldsymbol{G}_{\mathscr{B}\mathscr{C}}\,\boldsymbol{G}_{\mathscr{C}\mathscr{B}})^{r-1}\boldsymbol{G}_{\mathscr{B}\mathscr{C}}\,\boldsymbol{G}_{\mathscr{C}\mathscr{D}}\,u_{\mathscr{D}}/\boldsymbol{\phi}_{\mathrm{b}}\,\boldsymbol{Z}_{\mathscr{D}\mathscr{D}}^{m}\,u_{\mathscr{D}}. \end{aligned} \tag{58}$$

The mean is

$$\begin{aligned} \mathbb{E}(\tilde{r};m) &= \boldsymbol{\phi}_{\mathrm{b}}\,\boldsymbol{Z}_{\mathscr{D}\mathscr{D}}^{m-1}[\boldsymbol{G}_{\mathscr{F}\mathscr{B}}]_{\mathscr{D}\mathscr{B}}(\boldsymbol{I}-\boldsymbol{G}_{\mathscr{B}\mathscr{C}}\,\boldsymbol{G}_{\mathscr{C}\mathscr{B}})^{-2}\boldsymbol{G}_{\mathscr{B}\mathscr{C}}\,\boldsymbol{G}_{\mathscr{C}\mathscr{D}}\,u_{\mathscr{D}}/\boldsymbol{\phi}_{\mathrm{b}}\,\boldsymbol{Z}_{\mathscr{D}\mathscr{D}}^{m}\,u_{\mathscr{D}} \\ &= \boldsymbol{\phi}_{\mathrm{b}}[G_{\mathscr{F}\mathscr{B}}]_{\mathscr{D}\mathscr{B}}\,\boldsymbol{Z}_{\mathscr{B}\mathscr{B}}^{m-1}(\boldsymbol{I}-\boldsymbol{G}_{\mathscr{B}\mathscr{C}}\,\boldsymbol{G}_{\mathscr{C}\mathscr{B}})^{-2}\boldsymbol{G}_{\mathscr{B}\mathscr{C}}\,\boldsymbol{G}_{\mathscr{C}\mathscr{D}}\,u_{\mathscr{D}}/\boldsymbol{\phi}_{\mathrm{b}}\,\boldsymbol{Z}_{\mathscr{D}\mathscr{D}}^{m}\,u_{\mathscr{D}}. \end{aligned} \tag{59}$$

The probability of r openings in a burst regardless of the position of the burst in the run is

$$P(r) = \boldsymbol{\phi}_{\mathrm{b}}(\boldsymbol{I}-\boldsymbol{Z}_{\mathscr{D}\mathscr{D}})^{-1}[\boldsymbol{G}_{\mathscr{F}\mathscr{B}}]_{\mathscr{D}\mathscr{B}}(\boldsymbol{G}_{\mathscr{B}\mathscr{C}}\,\boldsymbol{G}_{\mathscr{C}\mathscr{B}})^{r-1}\boldsymbol{G}_{\mathscr{B}\mathscr{C}}\,\boldsymbol{G}_{\mathscr{C}\mathscr{D}}\,u_{\mathscr{D}}/\boldsymbol{\phi}_{\mathrm{b}}(\boldsymbol{I}-\boldsymbol{Z}_{\mathscr{D}\mathscr{D}})^{-1}\boldsymbol{Z}_{\mathscr{D}\mathscr{D}}\,u_{\mathscr{D}}, \tag{60}$$

with mean

$$\begin{aligned} \mathbb{E}(\tilde{r}) &= \boldsymbol{\phi}_{\mathrm{b}}(\boldsymbol{I}-\boldsymbol{Z}_{\mathscr{D}\mathscr{D}})^{-1}[\boldsymbol{G}_{\mathscr{F}\mathscr{B}}]_{\mathscr{D}\mathscr{B}}(\boldsymbol{I}-\boldsymbol{G}_{\mathscr{B}\mathscr{C}}\,\boldsymbol{G}_{\mathscr{C}\mathscr{B}})^{-2}\boldsymbol{G}_{\mathscr{B}\mathscr{C}}\,\boldsymbol{G}_{\mathscr{C}\mathscr{D}}\,u_{\mathscr{D}}/\boldsymbol{\phi}_{\mathrm{b}}(\boldsymbol{I}-\boldsymbol{Z}_{\mathscr{D}\mathscr{D}})^{-1}\boldsymbol{Z}_{\mathscr{D}\mathscr{D}}\,u_{\mathscr{D}} \\ &= \boldsymbol{\phi}_{\mathrm{b}}[\boldsymbol{G}_{\mathscr{F}\mathscr{B}}]_{\mathscr{D}\mathscr{B}}(\boldsymbol{I}-\boldsymbol{G}_{\mathscr{B}\mathscr{F}}\,\boldsymbol{G}_{\mathscr{F}\mathscr{B}})^{-1}[\boldsymbol{G}_{\mathscr{E}\mathscr{D}}]_{\mathscr{B}\mathscr{D}}\,u_{\mathscr{D}}/\boldsymbol{\phi}_{\mathrm{b}}(\boldsymbol{I}-\boldsymbol{Z}_{\mathscr{D}\mathscr{D}})^{-1}\boldsymbol{Z}_{\mathscr{D}\mathscr{D}}\,u_{\mathscr{D}}. \end{aligned} \tag{61}$$

The distribution of the total open time per run of single bursts

To define the fraction of time for which the channel is open during a run (which is an observable quantity) we need, for example, the distribution of the total open time per run. This is

$$f(t) = \boldsymbol{\phi}_{\mathrm{b}}[\boldsymbol{G}_{\mathscr{F}\mathscr{B}}]_{\mathscr{D}\mathscr{B}}\exp(\boldsymbol{V}_{\mathscr{B}\mathscr{B}}\,t)\,\boldsymbol{Q}_{\mathscr{B}\mathscr{C}}\,\boldsymbol{G}_{\mathscr{C}\mathscr{D}}\,e_{\mathrm{b}}/\boldsymbol{\phi}_{\mathrm{b}}\,\boldsymbol{Z}_{\mathscr{D}\mathscr{D}}\,u_{\mathscr{D}}, \tag{62}$$

where we define
$$\boldsymbol{V}_{\mathscr{B}\mathscr{B}} = \boldsymbol{Q}_{\mathscr{B}\mathscr{B}} + \boldsymbol{Q}_{\mathscr{B}\mathscr{F}}\,\boldsymbol{G}_{\mathscr{F}\mathscr{B}}. \tag{63}$$

The mean total open time per run of single bursts, μ_{to} say, is

$$\mu_{\mathrm{to}} = \boldsymbol{\phi}_{\mathrm{b}}[\boldsymbol{G}_{\mathscr{F}\mathscr{B}}]_{\mathscr{D}\mathscr{B}}(\boldsymbol{V}_{\mathscr{B}\mathscr{B}}^{-2})\boldsymbol{Q}_{\mathscr{B}\mathscr{C}}\,\boldsymbol{G}_{\mathscr{C}\mathscr{D}}\,e_{\mathrm{b}}/\boldsymbol{\phi}_{\mathrm{b}}\,\boldsymbol{Z}_{\mathscr{D}\mathscr{D}}\,u_{\mathscr{D}}. \tag{64}$$

Distribution of the number of individual openings per run of single bursts

The probability of r openings per run is

$$P_{\mathrm{o}}(r) = \boldsymbol{\phi}_{\mathrm{b}}[\boldsymbol{G}_{\mathscr{F}\mathscr{B}}]_{\mathscr{D}\mathscr{B}}(\boldsymbol{G}_{\mathscr{B}\mathscr{F}}\,\boldsymbol{G}_{\mathscr{F}\mathscr{B}})^{r-1}\boldsymbol{G}_{\mathscr{B}\mathscr{C}}\,\boldsymbol{G}_{\mathscr{C}\mathscr{D}}\,e_{\mathrm{b}}/\boldsymbol{\phi}_{\mathrm{b}}\,\boldsymbol{Z}_{\mathscr{D}\mathscr{D}}\,u_{\mathscr{D}}, \quad (r \geqslant 1), \tag{65}$$

with mean
$$\begin{aligned} \mathbb{E}_{\mathrm{o}}(\tilde{r}) &= \boldsymbol{\phi}_{\mathrm{b}}[\boldsymbol{G}_{\mathscr{F}\mathscr{B}}]_{\mathscr{D}\mathscr{B}}(\boldsymbol{I}-\boldsymbol{G}_{\mathscr{B}\mathscr{F}}\,\boldsymbol{G}_{\mathscr{F}\mathscr{B}})^{-2}\boldsymbol{G}_{\mathscr{B}\mathscr{C}}\,\boldsymbol{G}_{\mathscr{C}\mathscr{D}}\,e_{\mathrm{b}}/\boldsymbol{\phi}_{\mathrm{b}}\,\boldsymbol{Z}_{\mathscr{D}\mathscr{D}}\,u_{\mathscr{D}} \\ &= \boldsymbol{\phi}_{\mathrm{b}}[\boldsymbol{G}_{\mathscr{F}\mathscr{B}}]_{\mathscr{D}\mathscr{B}}(\boldsymbol{I}-\boldsymbol{G}_{\mathscr{B}\mathscr{F}}\,\boldsymbol{G}_{\mathscr{F}\mathscr{B}})^{-1}[\boldsymbol{G}_{\mathscr{E}\mathscr{D}}]_{\mathscr{B}\mathscr{D}}\,u_{\mathscr{D}}/\boldsymbol{\phi}_{\mathrm{b}}\,\boldsymbol{Z}_{\mathscr{D}\mathscr{D}}\,u_{\mathscr{D}}. \end{aligned} \tag{66}$$

Thus the mean number of openings per run is, as expected, the product of the mean number of bursts per run given by (47), and the mean number of openings per burst given by (61).

Distribution of the duration of individual openings in a run of single bursts

The overall distribution of the duration of any opening in a run of single bursts is

$$f(t) = \boldsymbol{\phi}_{\mathrm{b}}[\boldsymbol{G}_{\mathscr{F}\mathscr{B}}]_{\mathscr{D}\mathscr{B}}(\boldsymbol{I}-\boldsymbol{G}_{\mathscr{B}\mathscr{F}}\,\boldsymbol{G}_{\mathscr{F}\mathscr{B}})^{-1}\exp{(\boldsymbol{Q}_{\mathscr{B}\mathscr{B}}\,t)}(-\boldsymbol{Q}_{\mathscr{B}\mathscr{B}})[\boldsymbol{G}_{\mathscr{E}\mathscr{D}}]_{\mathscr{B}\mathscr{D}}\,\boldsymbol{u}_{\mathscr{D}}/\boldsymbol{\phi}_{\mathrm{b}}\,\boldsymbol{Z}_{\mathscr{D}\mathscr{D}}\,\boldsymbol{u}_{\mathscr{D}}.\mathbb{E}_{\mathrm{o}}(\tilde{r}), \tag{67}$$

with mean

$$\mu = \boldsymbol{\phi}_{\mathrm{b}}[\boldsymbol{G}_{\mathscr{F}\mathscr{B}}]_{\mathscr{D}\mathscr{B}}(\boldsymbol{I}-\boldsymbol{G}_{\mathscr{B}\mathscr{F}}\,\boldsymbol{G}_{\mathscr{F}\mathscr{B}})^{-1}(-\boldsymbol{Q}_{\mathscr{B}\mathscr{B}})^{-1}[\boldsymbol{G}_{\mathscr{E}\mathscr{D}}]_{\mathscr{B}\mathscr{D}}\,\boldsymbol{u}_{\mathscr{D}}/\boldsymbol{\phi}_{\mathrm{b}}\,\boldsymbol{Z}_{\mathscr{D}\mathscr{D}}\,\boldsymbol{u}_{\mathscr{D}}.\mathbb{E}_{\mathrm{o}}(\tilde{r}). \tag{68}$$

Distribution of shut times within a run of single bursts

By use of methods similar to those of Colquhoun & Hawkes (1982), the PDF of the duration of the kth shut time in a run of bursts with r openings is

$$\begin{aligned} f_{k,r}(t) = \boldsymbol{\phi}_{\mathrm{b}}[\boldsymbol{G}_{\mathscr{F}\mathscr{B}}]_{\mathscr{D}\mathscr{B}}(\boldsymbol{G}_{\mathscr{B}\mathscr{F}}\,\boldsymbol{G}_{\mathscr{F}\mathscr{B}})^{k-1}\boldsymbol{G}_{\mathscr{B}\mathscr{F}}\exp{(\boldsymbol{Q}_{\mathscr{F}\mathscr{F}}\,t)}(-\boldsymbol{Q}_{\mathscr{F}\mathscr{F}})\,\boldsymbol{G}_{\mathscr{F}\mathscr{B}}(\boldsymbol{G}_{\mathscr{B}\mathscr{F}}\,\boldsymbol{G}_{\mathscr{F}\mathscr{B}})^{r-k-1} \\ \boldsymbol{G}_{\mathscr{B}\mathscr{C}}\,\boldsymbol{G}_{\mathscr{C}\mathscr{D}}\,\boldsymbol{e}_{\mathrm{b}}/\boldsymbol{\phi}_{\mathrm{b}}\,\boldsymbol{Z}_{\mathscr{D}\mathscr{D}}\,\boldsymbol{u}_{\mathscr{D}}.P_{\mathrm{o}}(r), \quad (r \geqslant k+1). \end{aligned} \tag{69}$$

The overall distribution of shut times is

$$f(t) = \boldsymbol{\phi}_{\mathrm{b}}[\boldsymbol{G}_{\mathscr{F}\mathscr{B}}]_{\mathscr{D}\mathscr{B}}(\boldsymbol{I}-\boldsymbol{G}_{\mathscr{B}\mathscr{F}}\,\boldsymbol{G}_{\mathscr{F}\mathscr{B}})^{-1}\boldsymbol{G}_{\mathscr{B}\mathscr{F}}\exp{(\boldsymbol{Q}_{\mathscr{F}\mathscr{F}}\,t)}(-\boldsymbol{Q}_{\mathscr{F}\mathscr{F}})\,\boldsymbol{G}_{\mathscr{F}\mathscr{B}}[\boldsymbol{G}_{\mathscr{E}\mathscr{D}}]_{\mathscr{B}\mathscr{D}}\,\boldsymbol{u}_{\mathscr{D}}/d. \tag{70}$$

The normalizing factor, d, in the denominator is

$$\begin{aligned} d &= \boldsymbol{\phi}_{\mathrm{b}}[\boldsymbol{G}_{\mathscr{F}\mathscr{B}}]_{\mathscr{D}\mathscr{B}}(\boldsymbol{I}-\boldsymbol{G}_{\mathscr{B}\mathscr{F}}\,\boldsymbol{G}_{\mathscr{F}\mathscr{B}})^{-1}\boldsymbol{G}_{\mathscr{B}\mathscr{F}}\,\boldsymbol{G}_{\mathscr{F}\mathscr{B}}[\boldsymbol{G}_{\mathscr{E}\mathscr{D}}]_{\mathscr{B}\mathscr{D}}\,\boldsymbol{u}_{\mathscr{D}} \\ &= \boldsymbol{\phi}_{\mathrm{b}}\,\boldsymbol{Z}_{\mathscr{D}\mathscr{D}}\,\boldsymbol{u}_{\mathscr{D}}[\mathbb{E}_{\mathrm{o}}(\tilde{r})-1], \end{aligned} \tag{71}$$

where $[\mathbb{E}_{\mathrm{o}}(\tilde{r})-1]$ is the mean number of shut periods in the run.

The overall mean of shut times in the run is

$$\mu = \boldsymbol{\phi}_{\mathrm{b}}[\boldsymbol{G}_{\mathscr{F}\mathscr{B}}]_{\mathscr{D}\mathscr{B}}(\boldsymbol{I}-\boldsymbol{G}_{\mathscr{B}\mathscr{F}}\,\boldsymbol{G}_{\mathscr{F}\mathscr{B}})^{-1}\boldsymbol{G}_{\mathscr{B}\mathscr{F}}(-\boldsymbol{Q}_{\mathscr{F}\mathscr{F}})^{-1}\boldsymbol{G}_{\mathscr{F}\mathscr{B}}[\boldsymbol{G}_{\mathscr{E}\mathscr{D}}]_{\mathscr{B}\mathscr{D}}\,\boldsymbol{u}_{\mathscr{D}}/d. \tag{72}$$

The probability of being open during a run of single bursts

This can be calculated as the mean total open time in a run of single bursts, from (64), divided by the mean length of the run, from (39), *viz.*

$$P_{\mathrm{o2}} = \mu_{\mathrm{to}}/\mu_{\mathrm{r1}}. \tag{73}$$

This is the exact definition, and it would be experimentally appropriate too if the P_{open} were estimated by integration of the observed run, so omission of undetectably brief events would not cause problems. On the other hand, if P_{open} were estimated by fitting of individual durations, and if the bursts are clearly defined in the data, then it might be closer to experimental reality to calculate P_{open} as the mean total burst time per run, from (51), divided by the mean run length, from (39), *viz.*

$$P_{\mathrm{o2}} = \mu_{\mathrm{tb}}/\mu_{\mathrm{r1}}. \tag{74}$$

The numerical calculations presented next were all done by using the former definition, because when shut times within bursts of openings are brief there was little difference between the two definitions, and when openings are well-separated the former quantity can be measured accurately from the data.

Numerical results

Characteristics of runs of single openings, and of runs of single bursts, were calculated for a range of values of the rate constants, for each of the two mechanisms specified below. The 'observed' probability of being open during a run of single openings, P_{o2}, was calculated from (29) for runs of single openings, and from (73) for runs of single bursts. The agonist concentration, x_A, was adjusted iteratively (with a bisection method) to produce specified values of P_{o2} (e.g. 0.01, 0.025, 0.05, 0.1, 0.2, 0.3, 0.4).

For runs of single openings the initial vector defined in (9) was used for all calculations, but the other definitions of the initial vector gave very similar results. Calculations have been done for two different mechanisms, as follows.

Agonist mechanism with two sequential bindings

A commonly used mechanism postulates binding of two agonist molecules (A) to a shut receptor-channel (R), which may then isomerize to the open conformation (R^*), thus:

$$\begin{array}{lccccccc} & \mathrm{R} & \underset{k_{-1}}{\overset{2k_{+1}}{\rightleftharpoons}} & \mathrm{AR} & \underset{2k_{-2}}{\overset{k_{+2}}{\rightleftharpoons}} & \mathrm{A_2R} & \underset{\alpha}{\overset{\beta}{\rightleftharpoons}} & \mathrm{A_2R^*} \\ \text{state:} & 4 & & 3 & & 2 & & 1 \end{array} \tag{M1}$$

When two such channels are present there is one doubly open state (subset $\mathscr{A}$), three singly-open states (subset $\mathscr{B}$), and six shut states (subset $\mathscr{F}$). These states are numbered 1–10, and are specified in terms of the four underlying states defined in (M1) for a single channel, above each column of the $\boldsymbol{Q}$ matrix, which, when partitioned as defined by $\mathscr{A}$, $\mathscr{B}$, $\mathscr{C}$ and $\mathscr{D}$, is as follows (x_A denotes agonist concentration).

$$\begin{array}{c} \begin{array}{cccccccccc} 1 & 2 & 3 & 4 & 5 & 6 & 7 & 8 & 9 & 10 \\ (1,1) & (1,2) & (1,3) & (1,4) & (2,2) & (2,3) & (2,4) & (3,3) & (3,4) & (4,4) \end{array} \\ \boldsymbol{Q} = \left[\begin{array}{c|ccc|ccccc|c} q_{11} & 2\alpha & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ \hline \beta & q_{22} & 2k_{-2} & 0 & \alpha & 0 & 0 & 0 & 0 & 0 \\ 0 & k_{+2}x_A & q_{33} & k_{-1} & 0 & \alpha & 0 & 0 & 0 & 0 \\ 0 & 0 & 2k_{+1}x_A & q_{44} & 0 & 0 & \alpha & 0 & 0 & 0 \\ \hline 0 & 2\beta & 0 & 0 & q_{55} & 4k_{-2} & 0 & 0 & 0 & 0 \\ 0 & 0 & \beta & 0 & k_{+2}x_A & q_{66} & k_{-1} & 2k_{-2} & 0 & 0 \\ 0 & 0 & 0 & \beta & 0 & 2k_{+1}x_A & q_{77} & 0 & 2k_{-2} & 0 \\ 0 & 0 & 0 & 0 & 0 & 2k_{+2}x_A & 0 & q_{88} & 2k_{-1} & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & k_{+2}x_A & 2k_{+1}x_A & q_{99} & k_{-1} \\ \hline 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 4k_{+1}x_A & q_{10,10} \end{array}\right] \end{array}$$

The values of ten different sets of rate constants that were used for calculations with this mechanism are listed in table 2.

Table 2. Sets of parameter values for the agonist mechanism (M1)

(Values are in s^{-1} except for the association rate constants, which are in units of $M^{-1} s^{-1}$. The equilibrium constants, K_1 and K_2, are in μM.)

set	k_{+1}	k_{-1}	k_{+2}	k_{-2}	β	α	K_1	K_2
1	10^8	10^4	10^8	10^4	15100	1000	100	100
2	10^7	10^4	10^7	10^4	1.27×10^6	1000	1000	1000
3	10^9	10^4	10^9	10^4	500	1000	10	10
4	10^8	10^5	10^8	10^5	1.27×10^6	1000	1000	1000
5	10^9	10^4	10^7	10^4	37656.0	1000	10	1000
6	10^7	10^4	10^9	10^4	12929.7	1000	1000	10
7	10^8	10^5	10^8	10^3	12959.0	1000	1000	10
8	10^8	10^3	10^8	10^5	37695.3	1000	10	1000
9	10^8	10^3	10^8	10^3	500.8	1000	10	10
10	10^8	10^2	10^8	10^2	151.6	1000	1	1

For each set the agonist concentration was adjusted to produce the specified 'observed' probability of being open, P_{o2}, during a run of single openings with two channels.

Calculations in detail for $P_{o2} = 0.1$

The results of the calculations are summarized in some detail for the case where the channel is observed to be open for 10% of the time (i.e. $P_{o2} = 0.1$) in table 3. The characteristics of bursts of openings for one channel were calculated as described by Colquhoun & Hawkes (1982), with states 2 and 3 in (M1) being counted as 'within bursts' (subset $\mathscr{B}$), i.e., a burst corresponds to a single activation of the channel. The mean fraction of time for which an individual channel was open during a burst (i.e., total open time per burst/burst length) was calculated as an index of the extent to which the channel activation looks like a 'single opening', in the sense that interruptions within it are either very brief, rare, or both.

For runs of single openings with two independent channels the mean number of openings per run was calculated from (18). The mean length of the run, from the PDF in (14), was divided by the mean open time in the run (which is the observable quantity) from (24) to give the 'run length per unit open time'.

For runs of single bursts the mean length for all bursts in the run was calculated from (56), the distribution of all shut times from (70), and the mean number of bursts per run from (47). The mean run length from (39) was divided by the mean burst length to give the run length per unit burst length, in the last column of table 3.

The distribution of all shut times is given with one channel, and the distribution of shut times within a run of single bursts is given with two channels; the former has three components and the latter has six, but components that account for less than 1% of the area are omitted from table 3.

Distributions for $P_{o2} = 0.1$

It can be seen that a single channel must be open about 5.27% of the time to get runs of single openings that are open for 10.0% of the time. The mean open time for one channel, $1/\alpha$, was 1 ms in all cases; the distribution of open times in a run of single openings (or of single bursts), although it has three components, was always close to a single exponential with a shorter mean duration. It was about 0.95 ms for cases where openings are well-separated (close to the approximate value given in table 1). In such cases, the number of openings per burst may actually be larger than for one channel alone, because bursts in the two channels may overlap without producing a double opening, e.g. set 10 in table 3. On the other hand, when openings occur in bursts of many closely spaced openings, the mean open time for openings within a run are much the same (1 ms) as for one channel alone, but the mean burst length is reduced to about 95% of that for a single channel because the mean number of openings per burst is less than average for the bursts in the run (e.g. set 2 in table 3).

Agreement with the approximation for $P_{o2} = 0.1$

In some cases (e.g. set 3) the shut-time distribution consisted almost entirely of a single component, and there is essentially only one opening per burst; in such cases the number of openings per run of single openings, and the run length per unit open time, were close to the values given by the approximate calculation (18.8 and 188, respectively; table 1). The same is true for set 10; in this case there are several openings per burst but the openings are well-separated by quite long shut periods so individual bursts could not, in practice, be distinguished unambiguously from one another in the experimental record.

In other cases (when $\beta \gg k_{-2}$) individual channel activations had many brief interruptions and the distribution of shut times consisted predominantly of two components with very different time constants. For example set 2 gives 64.9 openings per burst (separated by gaps of 0.77 μs on average), with a mean separation between activations of 584 ms; in this case there are on average 1168 openings per run of single openings (far more than the approximation suggests); however there are 19.0 bursts per run of single bursts, exactly as predicted by the approximation. This is expected because the shut times within bursts are very brief, so two bursts that overlap are virtually certain to produce a double opening, i.e., a burst is an 'effective opening' for the purposes of these calculations (as indeed it is for physiological purposes).

In some cases (e.g. sets 8 and 9 in table 3), neither the number of openings per run of single openings, nor the number of bursts per run of single bursts, is very close to the prediction of the approximation. However, in both of these cases the shut-time distribution has three rather than two components with areas greater than 1%. For example set 8 gives 22.7 openings, or 15.8 bursts per run (compared with 18.8 from the approximation). In this case there are two components of gaps within bursts, one with a very short time constant (4.2 μs), and one much longer (0.45 ms). If we ignored all shut times associated with the 4.2 μs component (which would happen in practice because they would be too short to be detected) then there would appear to be 19.2 'effective openings' per run on average, which is very close to the value of 18.8 expected from the approximation.

It is clear from these, and the other examples, that the approximate calculation will provide a good estimate of the characteristics of runs of single openings as long as 'opening' is interpreted as meaning any group of consecutive openings that are

TABLE 3. CHARACTERISTICS OF SINGLE CHANNELS, AND OF RUNS OF SINGLE OPENINGS AND RUNS OF SINGLE BURSTS WITH TWO CHANNELS, FOR $P_{02} = 0.1$

(The agonist mechanism is specified in (M1), and the sets of rate constants for it are defined in table 2. Underlined values are close to the predictions of the approximation, *viz* 18.8 openings per run, and run length/opening length = 188 (see table 1). Values in parentheses give the factor by which the mean must be multiplied to get the value that would be exceeded in only 1% of cases (which would be 4.6 for a simple exponential distribution); e.g. for set 1, $P(\tilde{r} \geqslant 83.2) = 0.01$, as $4.5 \times 18.5 = 83.2$)

	ONE CHANNEL							TWO CHANNELS runs of single openings			TWO CHANNELS runs of single bursts					
					shut times:							shut times:				
set	P_{01} (%)	number of openings per burst	burst length/ ms	percent open in burst	mean/ ms	τ	area (%)	open time/ ms	number of openings per run	run length/ open time	burst length/ ms	mean/ ms	τ	area (%)	number of bursts per run	run length/ burst length
1	5.27	1.80	1.83	98.6	18.0	32.2 ms 88 µs 28 µs	55.9 2.6 41.5	0.970	32.7	327	1.75	9.00	16.1 ms 88 µs 28 µs	55.9 2.6 41.5	18.5 (4.5)	182.0 (4.8)
2	5.26	64.9	65.0	99.9	18.0	1172 ms 0.77 ms	1.5 98.5	0.999	1168	11684	61.6	9.00	584 ms 0.77 µs	1.5 98.5	19.0 (4.5)	190.0 (4.8)
3	5.26	1.04	1.04	99.6	18.0	18.4 ms 66 µs	97.8 1.4	0.948	19.4	193	1.02	9.00	9.20 ms 65 µs	97.8 1.4	18.2 (4.5)	180.0 (4.8)
4	5.26	7.39	7.40	99.9	18.0	133 ms 0.68 µs	13.5 86.4	0.993	134	1340	7.01	9.00	66.5 ms 0.68 µs	13.5 86.4	19.0 (4.5)	190.0 (4.8)
5	5.27	2.89	2.93	98.9	18.0	51.9 ms 17.3 µs	34.7 65.2	0.981	52.2	522	2.79	9.00	25.9 ms 17.3 µs	34.7 65.2	18.6 (4.5)	184.0 (4.8)
6	5.27	2.07	2.14	97.0	18.0	37.0 ms 92.9 µs 25.7 µs	48.5 25.3 26.2	0.974	36.9	369	2.03	9.01	18.5 ms 92.6 µs 25.7 µs	48.6 25.2 26.2	18.2 (4.5)	177.0 (4.8)
7	5.28	7.52	7.96	94.5	17.9	134 ms 66.9 µs	13.3 86.7	0.993	128	1280	7.52	9.00	67.2 ms 66.9 µs	13.3 86.7	17.9 (4.5)	169.0 (4.8)
8	5.27	1.29	1.38	94.0	18.0	21.8 ms 0.46 ms 4.2 µs	82.5 1.7 15.8	0.956	22.7	227	1.68	9.00	10.9 ms 0.45 ms 4.2 µs	82.5 1.7 15.8	15.8 (4.5)	129.0 (4.8)
9	5.28	1.38	1.67	82.3	17.9	22.0 ms 0.59 ms 0.31 ms	81.0 9.9 9.1	0.956	21.7	217	2.28	9.02	11.0 ms 0.57 ms 0.30 ms	81.2 9.8 9.0	13.6 (4.4)	90.9 (4.8)
10	5.35	2.95	15.5	19.0	17.7	24.4 ms 3.0 ms 1.8 ms	69.0 21.7 9.4	0.954	20.5 (4.5)	205 (4.8)	52.2	9.04	12.1 ms 2.7 ms 1.7 ms	68.2 22.0 9.2	2.76 (3.7)	3.71 (4.8)

separated by brief shut intervals. For example, 'openings' could be bursts of openings in rapid succession such that the channel is open for more than 95% of the time during the burst (so two overlapping bursts are virtually certain to produce a double opening).

Agreement with the approximation for P_{o2} values from 0.01 to 0.4

Results for other 'observed' P_{o2} values are summarized more briefly in table 4. Results are given in table 4 only for the mean number of bursts and of openings per run (the run length per unit burst length, or per unit opening length, follows similar lines). Clearly the approximate values in table 1 provide a sufficiently accurate guide for practical purposes whenever openings occur singly or in well-defined bursts. Furthermore the upper limit for the number of bursts per run, or for the run length as a multiple of burst length are given, to a sufficient degree of accuracy, by simply taking 4.6-times the mean value as the $P = 0.01$ limit, i.e. as the value that is likely to be exceeded by chance in 1 in 100 runs; the actual factor varies slightly according to the parameter values and P_{o2}, but is in the range 4.4–4.6 for the number of bursts per run, and 4.6–4.9 for the run length. Similarly 3.0–times the mean provides a reasonable limit for $P = 0.05$, and 6.9–times the mean is adequate for $P = 0.001$.

TABLE 4. AGONIST MECHANISM SPECIFIED IN (M1) WITH $P_{o2} = 0.01$ TO 0.4

(The mean number of bursts in a run of single bursts (bst/run) and the mean number of openings in a run of single openings (ops/run) for various values of the observable open probability, P_{o2}, for the sets of parameter values defined in table 2. Dashes indicate that the specified P_{o2} value cannot be attained at any agonist concentration.)

	value of P_{o2}													
	0.01		0.025		0.05		0.1		0.2		0.3		0.4	
set	bst/run	ops/run	bst/run	ops/run	bst/run	ops/run	bst/run	ops/run	bst/run	ops/run	bst/run	ops/run	bst/run	ops/run
1	196	347	77.7	138	38.2	67.7	18.5	32.7	8.6	15.1	5.3	9.25	3.7	6.3
2	199	12787	78.9	5044	39.0	2461	19.0	1168	9.0	521	5.7	305	4.0	197
3	198	204	78.3	80.9	38.3	39.9	18.2	19.4	8.0	9.12	4.1	5.7	1.7	4.0
4	199	1458	78.9	576	39.0	281	19.0	134	9.0	60.2	5.7	35.6	4.0	23.3
5	197	566	77.9	223	38.4	109	18.6	52.2	8.7	23.7	5.4	14.2	3.7	9.4
6	195	346	77.1	142	37.8	72.6	18.2	36.9	8.5	18.3	5.2	11.8	3.6	8.4
7	188	1403	74.6	553	36.8	270	17.9	128	8.4	57.1	5.3	33.4	3.7	21.5
8	195	238	75.2	94.6	35.5	46.7	15.8	22.7	6.1	10.6	3.2	6.60	2.0	4.6
9	182	235	69.5	92.9	32.2	45.5	13.6	21.7	4.5	9.87	1.8	5.97	1.1	4.1
10	113	236	36.0	92.3	12.1	44.3	2.76	20.5	1.0	9.09	—	—	—	—
approx.	199		79.0		38.9		18.8		8.7		5.2		3.4	

Clearly there are also intermediate cases where the shut times do not fall clearly into one, or two well-separated, components where the approximate calculation may not be a very good guide (fortunately such intermediate cases should be experimentally detectable by inspection of the distribution of shut times). For example sets 8, 9 and 10 of rate constants give bursts that contain relatively long shut times (see table 3). For set 10, and to a lesser extent set 9, the openings in the burst are so well separated that the number of openings per run is quite close to the approximation. For set 8, at first sight, neither the number of openings nor the number of bursts per run appears to be very close to the approximation at the larger P_{o2} values. However when the 4.2 μs component of shut times within bursts is ignored, as described above, the number of 'effective' openings per run becomes 19.2, 9.1, 5.7 and 4.0 for $P_{o2} = 0.1$, 0.2, 0.3 and 0.4, respectively, quite close to the predictions of the approximation. The characteristics of such intermediate cases are more easily explored by using the channel-block model, which is discussed in the next section.

Agonist mechanism with channel block

A simple version of a channel-block mechanism postulates that a blocking molecule, B, can enter and block the channel when it is open. To avoid increasing the total number of states a single agonist binding stage has been considered, thus:

$$\mathrm{R} \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} \mathrm{AR} \underset{\alpha}{\overset{\beta}{\rightleftharpoons}} \mathrm{AR}^* \underset{k_{-\mathrm{B}}}{\overset{k_{+\mathrm{B}}}{\rightleftharpoons}} \mathrm{AR^*B}. \tag{M2}$$

state: 4 2 1 3

When two such channels are present, with agonist concentration x_A and blocker concentration x_B, the $\boldsymbol{Q}$ matrix, again partitioned as defined by $\mathscr{A}$, $\mathscr{B}$, $\mathscr{C}$ and $\mathscr{D}$, is as follows.

$$\begin{array}{cccccccccc}
1 & 2 & 3 & 4 & 5 & 6 & 7 & 8 & 9 & 10 \\
(1,1) & (1,2) & (1,3) & (1,4) & (2,2) & (2,3) & (2,4) & (3,3) & (3,4) & (4,4)
\end{array}$$

$$\boldsymbol{Q} = \left[\begin{array}{c|ccc|ccccc|c}
q_{11} & 2\alpha & 2k_{+\mathrm{B}}x_\mathrm{B} & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
\hline
\beta & q_{22} & 0 & k_{-1} & \alpha & k_{+\mathrm{B}}x_\mathrm{B} & 0 & 0 & 0 & 0 \\
k_{-\mathrm{B}} & 0 & q_{33} & 0 & 0 & \alpha & 0 & k_{+\mathrm{B}}x_\mathrm{B} & 0 & 0 \\
0 & k_{+1}x_\mathrm{A} & 0 & q_{44} & 0 & 0 & \alpha & 0 & k_{+\mathrm{B}}x_\mathrm{B} & 0 \\
\hline
0 & 2\beta & 0 & 0 & q_{55} & 0 & 2k_{-1} & 0 & 0 & 0 \\
0 & k_{-\mathrm{B}} & \beta & 0 & 0 & q_{66} & 0 & 0 & k_{-1} & 0 \\
0 & 0 & 0 & \beta & k_{+1}x_\mathrm{A} & 0 & q_{77} & 0 & 0 & k_{-1} \\
0 & 0 & 2k_{-\mathrm{B}} & 0 & 0 & 0 & 0 & q_{88} & 0 & 0 \\
0 & 0 & 0 & k_{-\mathrm{B}} & 0 & k_{+1}x_\mathrm{A} & 0 & 0 & q_{99} & 0 \\
\hline
0 & 0 & 0 & 0 & 0 & 0 & 2k_{+1}x_\mathrm{A} & 0 & 0 & q_{10,10}
\end{array}\right]$$

The results of numerical calculations are summarized in table 5 for the case where the channel is observed to be present for 10 % of the time when two channels are active, i.e., $P_{o2} = 0.1$. The rate constants (given in the legend of table 5) have been chosen so that there are few spontaneous interruptions (*nachschlag* gaps) during channel activations, so virtually all of the interruptions arise from channel blockages. A range of mean blockage durations from 20 μs to 10 ms have been tested by appropriate choice of $k_{-\mathrm{B}}$. Table 6 gives briefer results for a range of values of P_{o2}.

It can be seen that when blockages are very brief (20 or 100 μs) the number of bursts (activations) per run, and the run length as a multiple of mean burst length, are well predicted by the simple approximations in table 1. At the other extreme when blockages are quite long (3 ms or 10 ms) so that individual openings are well separated (and bursts would, in any case, not be unambiguously distinguishable from each other in the experimental record) it can be seen (last two rows of tables 5 and 6) that the number of individual openings per run, and the run length

Table 5. Characteristics of single channels, and of runs of single openings and runs of single bursts with two channels for $P_{o2} = 0.1$

(Channel block mechanism (M2) with $k_{+1} = 10^9\ \text{M}^{-1}\ \text{s}^{-1}$, $k_{-1} = 10^5\ \text{s}^{-1}$, $k_{+B} = 5\times10^7\ \text{M}^{-1}\ \text{s}^{-1}$, $\beta = 1000$, $\alpha = 1000$, blocker concentration $x_B = 20\ \mu\text{M}$. Various values of k_{-B} from $5\times10^4\ \text{s}^{-1}$ to $100\ \text{s}^{-1}$ are tested; the corresponding values of mean blockage duration, $1/k_{-B}$, are given in the table. There are 2.02 openings per burst in all cases, and the mean open time (one channel) is $1/(\alpha + k_{+B}x_B) = 0.5$ ms. Other details are as in the legend of table 3. The approximate result (table 1) gives 18.8 openings per run, and run length/opening length = 188.)

	ONE CHANNEL			TWO CHANNELS runs of single openings			TWO CHANNELS runs of single bursts					
								shut times:				
$1/k_{-B}$	P_{o1} (%)	burst length/ ms	percentage open in burst	open time/ms	number of openings per run	run length/ open time	burst length/ms	mean/ms	τ	area (%)	number of bursts per run	run length/ burst length
20 μs	5.27	1.03	98.0	0.487	36.5	365	0.976	4.50	9.1 ms 20 μs	49.6 50.0	18.6 (4.5)	182
100 μs	5.29	1.11	90.9	0.486	33.9	339	1.05	4.51	9.0 ms 99 μs	49.7 49.9	17.3 (4.5)	157
0.316 ms	5.34	1.33	76.0	0.485	29.3	293	1.24	4.52	8.8 ms 0.31 ms	49.8 49.7	15.0 (4.4)	115
1.0 ms	5.43	2.02	50.0	0.481	23.6	236	1.82	4.53	8.3 ms 0.94 ms	49.2 49.6	12.0 (4.4)	61.8
3.16 ms	5.42	4.20	24.0	0.476	19.9 (4.5)	199	3.73	4.53	7.1 ms 2.6 ms 1.6 ms	44.7 49.6 5.2	9.1 (4.4)	24.5
10 ms	5.27	11.1	9.09	0.474	19.1 (4.5)	191	11.6	4.52	5.0 ms 4.5 ms 4.2 ms	22.5 49.6 27.5	5.5 (4.2)	7.1

TABLE 6. CHANNEL BLOCK MECHANISM SPECIFIED IN (M2) WITH $P_{o2} = 0.01$ TO 0.4

(The mean number of bursts in a run of single bursts (bst/run) and the mean number of openings in a run of single openings (ops/run) for various values of the observable open probability, P_{o2}, for the k_{-B} values specified in the first column. The other rate constants are as in the legend of table 5.)

	value of P_{o2}													
	0.01		0.025		0.05		0.1		0.2		0.3		0.4	
$1/k_{-B}$	bst/run	ops/run	bst/run	ops/run	bst/run	ops/run	bst/run	ops/run	bst/run	ops/run	bst/run	ops/run	bst/run	ops/run
20 μs	195	393	77.4	155	38.2	76.1	18.6	36.5	8.8	16.7	5.5	10.1	3.9	6.8
100 μs	182	367	72.3	145	35.6	70.9	17.3	33.9	8.1	15.4	5.1	9.2	3.5	6.1
316 μs	160	322	63.4	127	31.1	61.9	15.0	29.3	7.0	13.1	4.3	7.7	3.0	5.0
1.0 ms	132	265	51.8	104	25.2	50.4	12.0	23.6	5.4	10.3	3.3	6.1	2.3	4.1
3.16 ms	111	225	42.9	88.1	20.3	42.5	9.1	19.9	3.8	9.1	2.3	5.8	—	—
10.0 ms	98	207	36.0	81.1	15.4	39.4	5.5	19.1	—	—	—	—	—	—
approx.	199		79.0		38.9		18.8		8.7		5.2		3.4	

expressed as a multiple of the mean length of an individual opening, are given to a fair approximation by the approximate results in table 1.

However there are intermediate cases (middle two rows of tables 5 and 6) in which interruptions of the channel activations (0.3 ms or 1 ms) are of comparable duration to that of channel openings (*viz.* 0.5 ms in the presence of the blocker): in these cases neither of the above extreme cases is closely approached and the approximations in table 1 would not be a good guide. In these cases there is no fast component in the shut-time distribution elimination of which would give a number of effective openings per run that is close to the value predicted by the approximation. However examination of the shut time distributions shows that if shut times shorter than 80–400 μs (depending on the particular case) were undetected, the numbers of effective openings per run would be quite close to the approximate values. In any case these intermediate cases should be easily detectable in practice by inspection of the distribution of shut times during the run.

DISCUSSION

Detection of the presence of more than one channel

The results of exact calculations of the properties of runs of single openings or bursts when there are two independent channels functioning show that under certain circumstances the distributions of the length of such runs, and of the number of openings in the run, can be predicted with adequate accuracy by an approximate calculation based only on the observed properties of the run (fraction of time open, P_{o2}, and mean open time), without the need to know either the precise mechanism of channel function, or the rate constants for this mechanism. This is far more useful than the exact solution presented by Colquhoun & Hawkes (1983) because the latter was valid only under assumptions that are rarely fulfilled in practice. To the extent that the approximation holds, it is therefore possible to assess when an observed run of single openings is so long that it is highly unlikely that more than one channel is active.

The approximation is good under either of two conditions: (i) if openings occur singly so that the distribution of shut times during the run consists predominantly of a single component, or (ii) if channel activations consist of several openings (a burst) separated by short shut periods such that the channel is open for almost all of the time during a burst (which means that two overlapping bursts are virtually certain to produce a double opening). In the latter case, the term 'opening' in the approximate calculation is to be interpreted as a burst (which is, for physiological purposes too, just the 'effective opening'); the distribution of shut times within the run will consist predominantly of two components in this case, one with a very short time constant, and the other much longer. There is an intermediate class of cases in which the approximate calculation does not work well: these may have a more complex distribution of shut times within the run, with, for example intermediate duration component(s) as well as long and very brief components.

In many such cases, the number of 'effective' openings per run, that would result if the briefest shuttings within bursts were not detected (or were eliminated by imposition on the data of a realistic time resolution (Colquhoun & Sigworth 1983)), is quite close to the value predicted by the approximation. In any case such intermediate types of burst should be easily detectable by inspection of the distribution of shut times during the run, so erroneous conclusions should be avoidable.

Spurious correlations

It is pointed out that when there is more than one channel active there may be correlations between the length of one opening and the next during a run of single openings, even when there are no such correlations for a single channel. For all the numerical examples calculated here, the differences between the distributions of the duration of the 1st, 2nd, etc. openings in a run are so small that it is unlikely that they would be detectable in practice. Furthermore there are no correlations at all between the 1st, 2nd etc. bursts in a run for the examples used here, because all bursts are separated by one or more sojourns in the single $\mathscr{D}$ state. Nevertheless there is a theoretical possibility that the presence of more than one channel could lead to false conclusions about mechanism being drawn from measurements of correlations (see, for example, Fredkin *et al.* (1985); Colquhoun & Hawkes (1987)).

Overlapping clusters

It is common, in practice, for many sorts of channels to enter long-lived shut states that are variously described as 'desensitized', 'inactivated' or 'sleepy'. When all channels are in such states the result will be to generate long silent periods within the record that separate normal periods of activity (*clusters* of channel openings). Double openings may occur when there is overlap of two clusters that originate from different channels. However, clusters will obviously not overlap exactly, so if a cluster with no double openings is seen then the calculations suggested above cannot be applied as they stand because, in effect, there are two channels active for only part of the observed run. This is perhaps why the lengths of the runs of single openings calculated above may seem surprisingly short to those experienced at looking at single channel records.

The overlap of two clusters will be obvious if, as is usual at high P_{open}, double openings are seen. However, if no double openings are seen, overlap should (if it lasts long enough) be detectable as a period in the middle of the cluster during which P_{open} suddenly (almost) doubles. If such a run of openings with a relatively high P_{open} but no double openings is detected within a cluster, this run can be tested against the predictions made above; if it is much longer than could be reasonably expected on the basis that two independent channels are active then it might be that it may have resulted not from the presence of two channels, but, for example, from some biochemical modification of a single channel that increased temporarily the fraction of time for which that channel remains open.

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