

Chapter 18

The Principles of the Stochastic Interpretation of Ion-Channel Mechanisms

DAVID COLQUHOUN and ALAN G. HAWKES

1. The Nature of the Problem

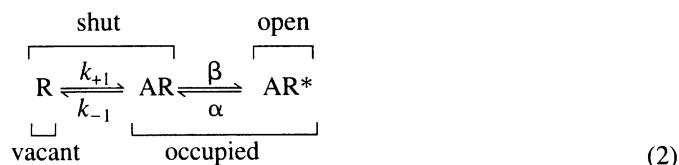
1.1. Reaction Mechanisms and Rates

Most mechanisms that are considered for ion channels (as for any other sort of chemical reaction) involve reversible transitions among the various possible discrete chemical states in which the system can exist. Other sorts of mechanisms may, of course, exist; for example, there may be an irreversible reaction step, a problem that is considered in Section 7. Our primary aim is to gain insight about the nature of the reaction mechanism from experimental observations. In this process, we may also obtain estimates of numerical values for the rate constants in the mechanism.

The sort of mechanism that is commonly considered can be illustrated by the following examples. In each case, the symbol that denotes the rate constant for a transition is appended to the arrow that represents the transition; the interpretation of these rate constants is considered below. The simplest reaction mechanism consists of a transition between a single shut state of the ion channel and a single open state:



There are two states altogether. If a ligand must be bound before the ion channel can open, at least three discrete states are needed to describe the mechanism. The mechanism of Castillo and Katz (1957) has two shut states and one open state; this is usually represented as



where R represents a shut channel, R* an open channel, and A represents the agonist molecule.

Note to the reader: At the author's request this chapter will use British spelling and the abbreviations ms and μ s instead of msec and μ sec.

DAVID COLQUHOUN • Department of Pharmacology, University College London, London WC1E 6BT, England, and ALAN G. HAWKES, European Business Management School, University of Wales Swansea, Swansea SA2 8PP, Wales.

Single-Channel Recording, Second Edition, edited by Bert Sakmann and Erwin Neher. Plenum Press, New York, 1995.

The simplified mechanism of the axonal sodium channel also has two discrete shut states and one open state; if inactivation of the channel can occur without channel opening, the reaction must be written in a cyclical form:



Some other examples are considered below (see Sections 4 and 13).

The usual procedure would be first to postulate a plausible mechanism, then to use the law of mass action to predict its expected kinetic and equilibrium behaviour, and finally to compare these predictions with experimental observations. Such predictions concern, of course, the *average* behaviour of the system.

If we are recording from a large number of molecules (ion channels, in the present case), then it is only the average behaviour that can be observed. For example, if the transition rates between the various states are constant (do not vary with time), then the time course of the mean current, $I(t)$, through the ion channels will be described by the sum of $k - 1$ exponential terms, where k is the number of states in the system (see examples above). Thus,

$$I(t) = I(\infty) + w_1 e^{-t/\tau_1} + w_2 e^{-t/\tau_2} + \dots \quad (4)$$

[Note that $\exp(-t/\tau)$ is often used as an alternative way of writing $e^{-t/\tau}$.] For any specified mechanism, the amplitudes w_i and the time constants τ_i can be calculated by the methods given, for example, by Colquhoun and Hawkes (1977), as can the predicted noise spectrum. A ‘cookbook’ approach to programming such calculations is provided in Chapter 20 (this volume). The values of τ_i each depend on *all* of the rate constants in the mechanism, and they have, in general, no simple physical significance (although in particular cases they may approximate some physical quantity such as mean open lifetime or mean burst length).

If, on the other hand, we record from a fairly small number of ion channels, the fluctuations about the average behaviour become large enough to measure, and Katz and Miledi (1970, 1972) showed how these fluctuations (or ‘noise’) could be interpreted in terms of the ion channel mechanism. Suppose, for example, that there are $N = 10^6$ ion channels and that, at equilibrium, there are 1000 channels open on average. The probability that an individual channel is open at a given moment is $p = 1000/10^6 = 0.001$, so the standard deviation of the number of open channels is given by the binomial distribution as $[Np(1-p)]^{1/2} = 31.6$. The number of channels that are open at equilibrium is therefore not constant at 1000, but is 1000 ± 31.6 , where the standard deviation reflects the random fluctuations in the number of open channels from moment to moment (see examples in Colquhoun, 1981, for an elementary discussion).

The law of mass action states that the rate of any reaction is proportional to the product of the reactant concentrations. The proportionality constant is described as a ‘rate constant’ (α , β , k_{-1} , etc.) and is supposed to be a genuine constant, i.e., not to vary with time. This is not necessarily true, however; for example, the channel-shutting rate constant, α , is known to be dependent on membrane potential (for muscle-type nicotinic receptors), so it will stay constant only if the membrane potential stays constant (i.e., only as long as we have an effective voltage clamp). Furthermore, for an association reaction with rate constant k_{+1} (dimension $M^{-1}s^{-1}$), the transition rate (dimensions s^{-1}) will be $k_{+1}x_A$ where x_A is the free

ligand concentration; this will be constant only if neither the ligand concentration nor the rate constant varies with time. In many sorts of experiments there is a considerable risk of transient concentration changes that would violate this condition. Of course, it is still possible to solve the kinetic equations even if the transition rates are not constant, as long as their time course is known, but this adds considerably to the complexity (we no longer get sums of exponential terms) and is not considered here.

We have already mentioned that the current, $I(t)$, is only an average value; there will be fluctuations about this average as a result of moment-to-moment random variations in the number of ion channels that are open. The smaller the number of ion channels that we record from, the larger (relative to the mean current) the fluctuations will be. When we record from a single ion channel, the current varies in a step-like fashion between (in the simplest cases) two values, fully open and fully shut (Neher and Sakmann, 1976; Hamill *et al.*, 1981). The current is effectively *never* equal to its equilibrium value; it is always zero or 100%. Equilibrium can be defined only over a long time period; the term “fraction of channels open at equilibrium” must be replaced by “the fraction of time for which the single channel stays open,” a quantity that can be measured accurately only over a period of observation long enough to contain many open and shut intervals. A long stretch of record is needed because we are looking at a single molecule, and its behaviour is, of course, random.

1.2. Rate Constants and Probabilities

In ordinary chemical kinetics, a rate constant describes the rate of reaction; for example, α in equation 1 or 2 describes the rate of the channel-shutting reaction. The transition rates (e.g., k_{-1} or $k_{+1}x_A$) have the dimensions of frequency (s^{-1}), and they can be interpreted as frequencies. For example, in equation 1, the number of shuttings that occur per second (of individual molecules) is simply α multiplied by the fraction of channels that are in the open state. At equilibrium, the number of shuttings per second (α times the fraction of channels that are open) will be equal, on average, to the number of openings per second (β' times the fraction of channels that are shut). This frequency interpretation of rate constants is described in more detail by Colquhoun and Hawkes (1994) and is illustrated in Sections 4.6 (Fig. 6) and 9.1.

However, when we look at a single ion channel, we see that the shutting takes place at random, so the rate constant must be interpreted in a probabilistic way: α is a measure of the probability that an open channel will shut in unit time (though, because α has dimensions of s^{-1} , it is clearly not an ordinary probability, which must be dimensionless). Roughly, we can say that for a time interval Δt ,

$$\text{Prob(open channel shuts during } \Delta t) \cong \alpha \Delta t$$

This is dimensionless, but it is still not a proper probability because it can be greater than unity. Also, this definition does not make clear whether or not several openings and shuttings are allowed to occur during the time interval Δt . It turns out that the proper way to write this definition can be arrived at by introducing a ‘remainder term,’ which we do not specify in detail but which has the property that it disappears (relative to Δt) as Δt becomes very small. This term would describe, for example, the possibilities of several transitions occurring during Δt , which clearly becomes negligible for small Δt . This remainder

term is written as $o(\Delta t)$ (further discussion of such terms is found, for example, in Colquhoun, 1971, Appendix 2). Thus, we can now write:

$$\text{Prob(open channel shut during } \Delta t) = \alpha \Delta t + o(\Delta t)$$

More properly, the left-hand side should be written as a conditional probability, the probability that a channel shuts during Δt given that it was open at the start of this period:

$$\text{Prob(channel shuts between } t \text{ and } t + \Delta t | \text{channel was open at } t) = \alpha \Delta t + o(\Delta t) \quad (5)$$

(the vertical bar is read as “given”; see Section 2).

Notice that this is supposed to be the same at whatever time t we start timing our interval, and also to be independent of what has happened earlier, i.e., it depends only on the present (time t) state of the channel. This is a fundamental characteristic of our type of random process (a homogeneous Markov process). Further progress appears to be prevented by the unspecified term $o(\Delta t)$ in equation 5. This term can, however, always be eliminated by dividing by Δt and then letting Δt tend to zero. In this way, the $o(\Delta t)$ terms disappear, and we obtain a differential equation that can be solved in the normal way. This procedure is illustrated in Section 3.

We have defined a probability in terms of a rate constant in equation 5; rearrangement of this gives a definition of the rate constant in probabilistic terms. Roughly,

$$\alpha \approx (\text{Probability of shutting in } \Delta t) / \Delta t$$

or, more properly, from equation 5,

$$\begin{aligned} \alpha &= \lim_{\Delta t \rightarrow 0} [\text{Prob(channel shuts between } t \text{ and } t + \Delta t | \text{open at } t) / \Delta t] \\ &= \lim_{\Delta t \rightarrow 0} [\text{Prob(channel shut at } t + \Delta t | \text{open at } t) / \Delta t]. \end{aligned} \quad (6)$$

More generally, we can define any transition rate in this way. Denote as q_{ij} the transition rate from state i to state j . Then

$$\begin{aligned} q_{ij} &= \lim_{\Delta t \rightarrow 0} [\text{Prob(state } j \text{ at time } t + \Delta t | \text{state } i \text{ at } t) / \Delta t] \quad i \neq j \\ &= \lim_{\Delta t \rightarrow 0} [P_{ij}(\Delta t) / \Delta t] \end{aligned} \quad (7)$$

where

$$P_{ij}(t) = \text{Prob(state } j \text{ at time } t | \text{state } i \text{ at time } 0) \quad (8)$$

Notice that a transition rate must always have dimensions of s^{-1} .

1.3. Fractal and Diffusion Models

Classical chemical kinetics, based on the law of mass action, has always entailed the assumption that the system can exist in a small number of discrete states, as in the examples

given above. The advent of single-channel recording has provided perhaps the strongest and most direct evidence for the existence of discrete states of large protein molecules. The switch between shut and open states, or between open states of different conductance, is very fast, with no detectable intermediate states; this is exactly what is expected on the basis of the classical postulates of chemical kinetics. Yet, ironically, it is these same observations that have caused these postulates to be questioned.

It has been suggested that, because proteins can exist in an essentially infinite number of conformations, it is inappropriate to postulate a small number of discrete states, and some sort of fractal or diffusion model should be preferred (Liebovitch *et al.*, 1987; Läuger, 1988; Milhauser *et al.*, 1988; Liebovitch, 1989). Such models usually predict that the probability of a transition occurring in unit time will not be constant and so differ fundamentally from the Markov model. There are several reasons to think that such approaches are not, at present, likely to be very helpful.

The most important reason is that the experimental evidence shows Markov models to fit the data better than the alternatives (as formulated up to now); see, for example Korn and Horn (1988), McManus *et al.* (1988), Sansom *et al.* (1989), McManus and Magleby (1989), Petracchi *et al.* (1991), and Gibb and Colquhoun (1992). An example of this evidence is given later (see Section 10.3).

A second reason is that the theoretical argument is not entirely convincing. Fractals stem from mathematics rather than physics, so it is far from clear what they can tell us about the real world (the same comment applies to catastrophe theory, which, at the height of its fashion, was said to "explain" almost every biological phenomenon from riots to action potentials but is now almost forgotten). Diffusion theory, on the other hand, has a sound physical basis and must be taken more seriously. Clearly, a protein (or, indeed, much smaller molecules) can exist in an infinite number of conformations, but this does not preclude the existence of a limited number of states, or conformations, that are much more stable than the others (e.g., Läuger, 1985, 1988). Such "discrete" states are not, of course, fixed and stationary. All the parts of the molecule have thermal motion, much of it very rapid (on a picosecond time scale), so there is continuous fluctuation around the average structure of the "discrete" state, but if these fluctuations are of no great functional significance (e.g., have only a small effect on channel conductance), then there is no need to incorporate them into the model. To attempt to do so merely increases vastly the number of parameters to be estimated without contribution to the usefulness of the model. In fact, the fractal formulation does not attempt the impossible task of estimating all the relevant parameters but, on the contrary, attempts to describe the data with only two, neither of which has any obvious physical significance.

In summary, the simple forms of the fractal argument that have been used fail to fit the data adequately in many cases. Furthermore, even if it were true that an infinite (or at least very large) number of states should be considered, it would be impossible to estimate from experimental data parameters with genuine physical significance. Just as in all other science, the mechanisms with a few discrete states that we use are undoubtedly approximations, but they have parameters that can be estimated, have real physical significance, and they have proven predictive value (see Colquhoun and Ogden, 1986; Horn and Korn, 1989).

2. Probabilities and Conditional Probabilities

The definitions just given are in terms of probabilities and conditional probabilities. It may be useful at this stage to illustrate exactly what these terms mean. Consider the behavior

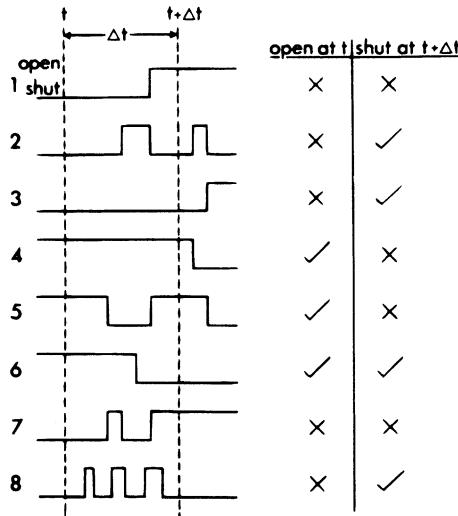


Figure 1. Illustration of the meaning of probabilities and conditional probabilities (see text).

of eight individual ion channels illustrated in Fig. 1. Imagine that these eight channels behave in a manner typical of a much larger number of channels so that the ratios given are good estimates of the true or long-term average values of the probabilities. One possible example of their behaviour is shown in Fig. 1.

Only one channel of the eight is both open at t and shut at $t + \Delta t$, so

$$\begin{aligned} \text{Prob(open at } t \text{ and shut at } t + \Delta t) &= (\text{number open at } t \text{ and shut at } t + \Delta t) / (\text{total number}) \\ &= 1/8. \end{aligned}$$

However, the conditional probability, $\text{Prob(shut at } t + \Delta t | \text{open at } t)$, although it has the same numerator, has a different denominator; it is defined with respect to the population of channels that obey the prior condition, i.e., those that were open at t . These are three in number (channels 4, 5, and 6), so the conditional probability is

$$\begin{aligned} \text{Prob(shut at } t + \Delta t | \text{open at } t) &= (\text{number open at } t \text{ and shut at } t + \Delta t) / (\text{number open at } t) \\ &= 1/3. \end{aligned}$$

This is an example of the general rule of probability that for any events A and B,

$$\begin{aligned} \text{Prob}(B | A) &= \text{Prob}(A \text{ and } B) / \text{Prob}(A) \\ \text{Prob}(A \text{ and } B) &= \text{Prob}(A) \text{ Prob}(B | A) \end{aligned} \tag{9}$$

In this case, A is ‘open at t '; B is ‘shut at $t + \Delta t$.' So in this example,

$$\text{Prob}(B | A) = (1/8) / (3/8) = 1/3$$

In general, if A and B are *independent*, then the probabilities of B cannot depend on whether A has occurred or not. Therefore, $\text{Prob}(B \mid A)$ can be written simply as $\text{Prob}(B)$, and equation 9 reduces to the simple multiplication rule:

$$\text{Prob}(A \text{ and } B) = \text{Prob}(A) \text{ Prob}(B) \quad (10)$$

3. The Distribution of Random Time Intervals

3.1. The Lifetime in an Individual State

We are interested in the length of time for which the system stays in a particular state, for example, the open state. These lengths of time are random variables, and the form of their variability can be described by a probability distribution. Time is a continuous variable, so we wish to find the probability density function (pdf) of, for example, open lifetimes. This is a function $f(t)$, defined so that the area under the curve up to a particular time t represents the probability that the lifetime is equal to or less than t . Thus, the pdf can be found by differentiating the cumulative distribution (or *distribution function*) $\text{Prob}(\text{open lifetime} \leq t)$, which is usually denoted $F(t)$. The pdf is thus

$$f(t) = \lim_{\Delta t \rightarrow 0} [\text{Prob}(\text{lifetime is between } t \text{ and } t + \Delta t) / \Delta t] \quad (11)$$

A number of approaches to the derivation of this distribution are possible. We shall first derive it directly and then mention some other approaches. Take, as an example, the length of time for which a channel stays open. First define a probability, which we shall denote $R(t)$, as

$$R(t) = \text{Prob}(\text{channel stays open throughout the time from 0 to } t) \quad (12)$$

It is worth noting that this is a rather different sort of probability from that used in analyzing relaxations or noise. In these cases, we are interested, for example, in the probability that a channel is open at time t , given that it was open at $t = 0$, regardless of whether the channel may have shut one or more times in between. In reliability theory, the sort of probability defined in equation 12 is known as the *reliability function*; it represents the probability that a system remains operational throughout the period 0 to t .

Now, from equation 5 we know that

$$\text{Prob}(\text{shut at } t + \Delta t \mid \text{open at } t) = \alpha \Delta t + o(\Delta t), \quad (13)$$

where α is the ordinary rate constant for the shutting reaction (or, more generally, the sum of the rate constants for all routes by which the open channel can shut). The channel obviously must either shut or not shut during Δt , so the probabilities for these two alternatives must add to unity. Hence,

$$\text{Prob}(\text{channel does not shut between } t \text{ and } t + \Delta t \mid \text{open at } t) = 1 - \alpha \Delta t - o(\Delta t), \quad (14)$$

From equations 9 and 12, we can now define

$$\begin{aligned} R(t + \Delta t) &= \text{Prob(channel stays open throughout the time from 0 to } t + \Delta t) \\ &= \text{Prob(open throughout } 0, t) \cdot \text{Prob(open throughout } t, t + \Delta t | \text{open} \\ &\quad \text{throughout } 0, t) \end{aligned} \quad (15)$$

Now the crucial Markov assumption, discussed following equation 5, is that the last probability does not depend on the whole history from 0 to t but only on the fact that the channel is open at time t . Thus,

$$\begin{aligned} &\text{Prob(open throughout } t, t + \Delta t | \text{open throughout } 0, t) \\ &= \text{Prob(open throughout } t, t + \Delta t | \text{open at } t) \end{aligned} \quad (16)$$

But equation 16 is just the probability that was derived in equation 14, and the first probability in equation 15 is simply $R(t)$, so equation 15 can be written as

$$R(t + \Delta t) = R(t)[1 - \alpha\Delta t - o(\Delta t)] \quad (17)$$

Thus,

$$\frac{R(t + \Delta t) - R(t)}{\Delta t} = -R(t) \left[\alpha + \frac{o(\Delta t)}{\Delta t} \right]$$

If we now let $\Delta t \rightarrow 0$, the left-hand side becomes the first derivative of $R(t)$, and the remainder term disappears, so

$$\frac{dR(t)}{dt} = -\alpha R(t). \quad (18)$$

As long as α is a constant (not time dependent), the solution of this equation is

$$R(t) = e^{-\alpha t} \quad (19)$$

because $R(0) = 1$ (i.e., channel cannot move out of the open state in zero time).

Next, we notice that if the channel stays open throughout the time from 0 to t , its open lifetime must be at least t . This is the crucial step that relates the argument to the distribution of open times. We can therefore write

$$\begin{aligned} R(t) &= e^{-\alpha t} = \text{Prob(channel stays open throughout time from 0 to } t) \\ &= \text{Prob(open lifetime} > t) \end{aligned} \quad (20)$$

and therefore,

$$\text{Prob(open lifetime} \leq t) = 1 - R(t) = 1 - e^{-\alpha t} = F(t) \quad (21)$$

This defines the cumulative distribution, $F(t)$, of open-channel lifetimes. The required pdf for the open-channel lifetime is the first derivative of this, i.e.,

$$f(t) = dF(t)/dt = -\frac{dR(t)}{dt} = \alpha e^{-\alpha t} \quad (t \geq 0) \quad (22)$$

[for times less than zero, $f(t) = 0$].

This pdf is described as an *exponential distribution*, or exponential density, with mean $1/\alpha$. It is a simple exponentially decaying curve. This is quite different in shape from the well-known Gaussian or “normal” distribution: rather than being a symmetrical bell-shaped curve, it is an extreme example of a positively skewed distribution with the mode (maximum) at $t = 0$ (compare with the Gaussian curve for which the mode is the same as the mean). The exponential distribution has the same sort of central role in stochastic processes as the Gaussian distribution has in large areas of classical statistics.

For any pdf, $f(t)$, the mean is given by

$$\text{mean} = \int_{-\infty}^{\infty} tf(t)dt$$

For nonnegative random variables, $f(t) = 0$ when $t < 0$, and the lower limit of this integral can be taken as zero; then integration by parts yields a useful alternative formula that is sometimes easier to calculate. Thus,

$$\text{mean} = \int_0^{\infty} tf(t)dt = \int_0^{\infty} R(t)dt \quad (23)$$

which, in the present example, is $1/\alpha$, the mean open lifetime.

3.2. Another Approach to the Exponential Distribution

An open channel must overcome a certain energy barrier before it can flip to a shut conformation. The energy needed for this purpose comes from the random thermal energy of the system. The bonds of the channel protein will be vibrating, bending, and stretching, and much of this motion will be very rapid, on a picosecond time scale. One can imagine that each time the molecule stretches, it has a chance to surmount the energy barrier and flip shut. Each “stretch” is like a binomial trial with a certain probability, p , of success (i.e., shutting) at each trial. Since the stretching is on a picosecond time scale, but the channel stays open for milliseconds, clearly, the chance (p) of success at each trial must be small, and a large number (N) of trials will be needed before the channel shuts. Now, when N is large and p is small, the binomial distribution approaches the Poisson distribution. The Poisson distribution gives the probability of there being no successes (i.e., no shutting) in time t as $e^{-\alpha t}$, where α is the mean frequency of successes in unit time. If there is no success at shutting in time t , then the open lifetime must be greater than t , and, since we have found that the probability of this is $e^{-\alpha t}$, we are led directly to equation 20 and hence to the exponential pdf.

3.3. Generalizations

The above argument was mostly concerned with the open time, but clearly the same argument applies to the time spent in any single specified state. For the simplest mechanism (equation 1), therefore, the open time is exponentially distributed with mean $1/\alpha$, and the shut time has pdf $g(t) = \beta' e^{-\beta' t}$, i.e., it is exponentially distributed with mean $1/\beta'$ for a single channel. If more than one channel contributes to the observations, the shut times will appear to be shorter than this, of course. In general, we can, by a similar argument, give the following rule:

$$\begin{aligned} \text{Lifetime in any single state is exponentially distributed with mean} \\ = 1/(\text{sum of transition rates that lead away from the state}) \end{aligned} \quad (24)$$

In general, for mechanisms with many states, we expect that all of the distributions of quantities such as open times, shut times, burst lengths, and so on will be mixtures of exponentials. This will be the case when the transition rates are constant (see above), and we therefore expect Markov behaviour. Such distributions are often described as being a *sum of exponential components*, just like the macroscopic current in equation 4 (except that there will usually be fewer components in the single-channel distributions). It is actually preferable to refer to such distributions as having the form of a *mixture of exponential distributions* (or of exponential densities). Each component can be written in the form of a simple exponential distribution, i.e., $\lambda_i \exp(-\lambda_i t)$, where λ_i is the reciprocal of the time constant, or mean, for the i th component, $\tau_i = 1/\lambda_i$. Each such distribution has unit area, and to ensure that the final distribution also has unit area, each component is multiplied by a fraction area, a_i , the relative area occupied by the i th component; these are such that the sum of the areas is unity. Thus, the general form for a pdf that is a mixture of exponentials is

$$f(t) = a_1 \lambda_1 e^{-\lambda_1 t} + a_2 \lambda_2 e^{-\lambda_2 t} + \dots,$$

or, for n components,

$$f(t) = \sum_{i=1}^{i=n} a_i \lambda_i e^{-\lambda_i t} \quad (25)$$

where

$$\sum a_i = 1.$$

The question of the number of components that would be expected in particular cases is addressed below and discussed more generally in Section 13.5.

It is often of interest to know the distribution of the time spent within any specified set of states (e.g., all shut states) rather than in a single state. In this case the system can oscillate among the various states within the set in a random way; the time that elapses before the set of states is eventually left will (under our usual assumptions) be described by a mixture of exponential distributions. The derivation of such distributions is exemplified by the burst-length distribution discussed in Section 4.7 and by the derivation of the shut-time distribution

given by Colquhoun and Hawkes (1994). The general solution is given by Colquhoun and Hawkes (1982), and this is discussed briefly in Section 13.3 and Chapter 20 (this volume).

3.4. Relationship between Single-Channel Events and Whole-Cell Currents

It is, of course, no coincidence that, on one hand, the mean current through a large number of ion channels follows an exponential time course (see equation 4) and, on the other hand, the random lifetimes of elementary events are described by exponential distributions. This can be illustrated schematically for the case of the decay phase of a miniature end-plate current. According to Anderson and Stevens (1973), the decay phase, which follows a simple exponential time course, is determined by the lifetime of individual open channels. This is illustrated in Fig. 2b,c. At zero time, a number of ion channels are opened, almost

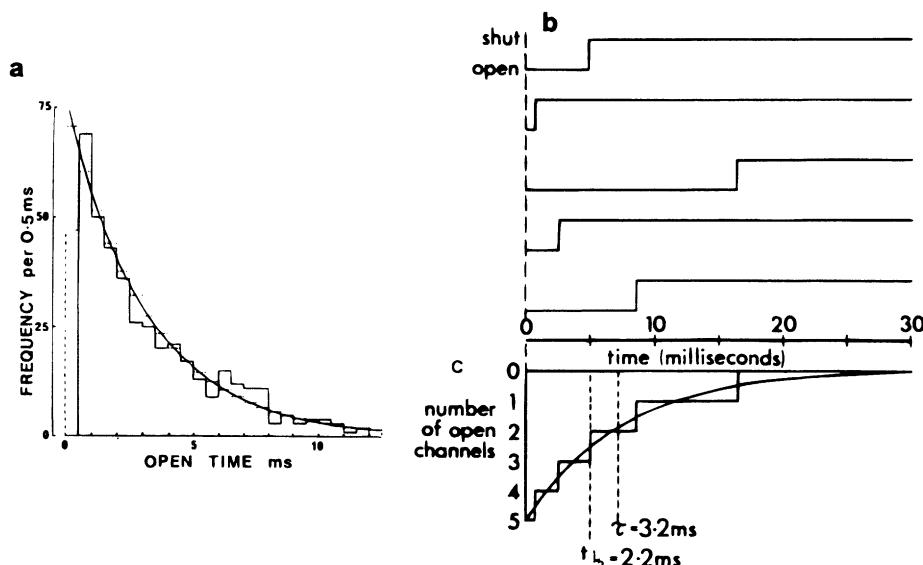


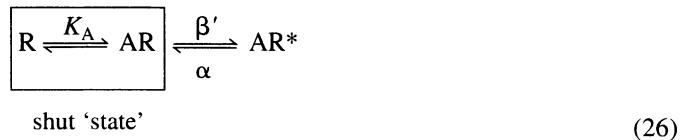
Figure 2. a: An exponential distribution of the duration of channel-open times. The histogram shows the number of openings per bin of 0.5-msec width (*R. temporaria*, synaptic channels, 50 nM acetylcholine, -80 mV, 8°C; D. C. Ogden, D. J. Adams, and D. Colquhoun, unpublished data). The continuous line shows an exponential probability density function that has been fitted to the observations (above 0.5 ms) by the method of maximum likelihood (see Chapter 19, this volume). It has a time constant of $\tau = 3.2 \text{ ms}$, i.e., a rate constant of $\lambda = 1/\tau = 312.5 \text{ s}^{-1}$. The estimated exponential probability density function is therefore (see equation 22) $f(t) = \lambda e^{-\lambda t} = 312.5 e^{-312.5 t} \text{ s}^{-1}$, the area under this curve is, as for any probability density function, unity. In the figure, $f(t)$ has been multiplied by the number of observations that lie under the fitted curve (480) and expressed in units of $(0.5 \text{ ms})^{-1}$ rather than s^{-1} , so the continuous curve can be superimposed on the histogram (see Section 5.1.5 of Chapter 19, this volume). Thus, for example, the intercept at $t = 0$ is plotted not as $f(0) = 312.5 \text{ s}^{-1}$ but as $312.5 \text{ s}^{-1} \times 480/2000 = 75 (0.5 \text{ ms})^{-1}$, where the factor 2000 is 0.5 ms/1s. The horizontal dashed lines show the frequency in each bin as calculated from the continuous curve. b: Simulated behaviour of five individual channels that were open at the time ($t = 0$) at which the acetylcholine concentration had fallen to zero. Opening is plotted downward. The channels stay open for a random (exponentially distributed) length of time with a mean of 3.2 ms. c: Sum of the five records shown in b. The total number of open channels decays exponentially (as illustrated in Fig. 3c) with a time constant of 3.2 ms in this example. Reproduced from Colquhoun (1981), with permission.

synchronously, by a quantum of acetylcholine; the acetylcholine then rapidly disappears so that a channel, once it has shut, cannot reopen. The length of time for which each channel stays open is described by an exponential distribution (Fig. 2a,b), which ensures that the total current through a large number of such channels will decay along an exponential time course (Fig. 2c).

This simple argument works only because the channels were supposed to open almost simultaneously. This is true, to a good approximation, for synaptic transmission mediated by nicotinic receptors, but it is far from true for NMDA-type glutamate receptors (Edmonds and Colquhoun, 1992). In such cases we need also to consider the distribution of the time (first latency) from the application of the stimulus (e.g., synaptic release of transmitter) to the time when the channel first opens. The complications that arise in such cases will be considered in Sections 9–11.

3.5. Pooling States That Equilibrate Rapidly

If, in mechanism 2, the binding step were very fast compared with the subsequent conformation change, and so fast that it was beyond the resolution of the experiment, then the vacant and occupied states would behave, experimentally, as a single (shut) state. This may be represented diagrammatically by enclosing the two states in a box, thus:



If the binding and dissociation are fast enough, the vacant and occupied states will be close to equilibrium at all times (even if the system as a whole is not). Therefore, the transition between them has been labelled only with the equilibrium constant, $K_A = k_{-1}/k_{+1}$, rather than with the separate rate constants. This procedure has reduced the effective number of states in the mechanism from three to two (just shut and open). This does not affect the way we look at the shutting reaction, with rate constant α . However, we have to be more careful about how we treat the opening reaction. The transition rate from shut to open can no longer be taken as β , because the “shut state” spends part of its time without ligand bound (R), and while the receptor is not occupied, opening is impossible. The fraction of time for which the “shut state” is occupied (in AR) and so capable of opening is simply the equilibrium fraction of shut states that are occupied, i.e., $x_A/(x_A + K_A)$. Thus, the effective opening rate constant is

$$\beta' = \beta \left(\frac{x_A}{x_A + K_A} \right) \quad (27)$$

When β' is so defined, the three-state mechanism in equation 2 becomes formally identical to the two-state mechanism in equation 1.

The argument just presented is related to the discussion of ‘discrete states’ in Section 1.3. What we refer to here as a discrete state must, since the protein is not stationary, consist of many conformations (or substates) that interchange rapidly. But if the lifetime of each

individual substate is exponentially distributed, we expect that the overall lifetime will itself be a mixture of exponentials with Markovian behaviour. If most of the time is spent in one substate, which may be visited many times, then we expect that the distribution of the overall lifetime will itself be essentially a simple exponential. This is a result of the following fact, which is derived later, in Section 9.3:

$$\text{The sum of a random number of exponentially distributed time intervals is itself exponentially distributed.} \quad (28)$$

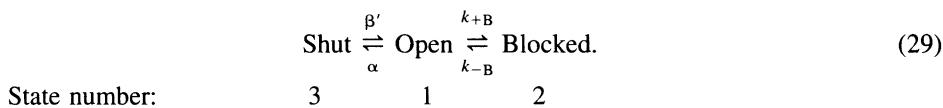
What we mean by a “fast” reaction depends entirely on the time resolution of the experiment as well as on the rates of other steps in the mechanism. What is fast in one context may be slow in another; further examples are given by Colquhoun and Hawkes (1994).

4. A Mechanism with More Than One Shut State: The Simple Open Ion Channel-Block Mechanism

The two-state mechanism in equation 1 is simple because it is possible to tell which of the two states the system is in at any moment simply by inspecting the experimental record (though, in practice, complications would arise if more than one channel were contributing to the recording; see Section 8). In most cases of practical interest, there are likely to be several (experimentally indistinguishable) shut states, and possibly more than one open state too (see, for example, Section 13). It may be noted that, insofar as there will usually be more shut states than open ones, the distributions of shut periods are potentially far more informative than the distributions of open times. A simple example of a mechanism with two shut states is now considered in some detail.

4.1. A Simple Ion Channel-Block Mechanism

Consider the following simple mechanism (Armstrong, 1971; Adams, 1976) for ion channel block, which assumes that agonist binding is much faster than the open–shut reaction, as discussed in Section 3.5 (this is unlikely to be true, at least for the muscle nicotinic receptor).



In this mechanism, the transition rate from open to blocked states is $k_{+B}x_B$, where x_B is the concentration of the blocking molecule. In this example, there are two shut states (shut and blocked). Neither of the shut states conducts any current, so it is not possible to tell for certain which of the two shut states the system is in at any moment simply by looking at the experimental record. This makes matters more complicated.

4.2. Relaxation and Noise

In this example, there are $k = 3$ states, so it would be expected that relaxations and noise experiments would be described by the sum of two components (exponential or Lorentzian,

respectively) with rate constants denoted λ_1 and λ_2 . The following results can be derived as described by Colquhoun and Hawkes (1977).

Although it is often convenient to derive results in terms of the rate constants λ_1 and λ_2 , it is preferable, whenever possible, to refer to the time constants, $\tau_1 = 1/\lambda_1$ and $\tau_2 = 1/\lambda_2$ (as used in equation 4). There are two reasons for this. First, it avoids confusion between the *fundamental rate constants* in the mechanism (k_{-1} , etc), and the derived or *observed rate constants*, λ . Each of the observed rate constants depends on *all* of the fundamental rate constants. These components are easy to observe in the case of some channel-blocking drugs, as illustrated in Fig. 3. Second, it is easier to think in terms of time rather than frequency.

In this case, we find the two rate constants to be the solutions of the quadratic equation

$$\lambda^2 + b\lambda + c = 0 \quad (30)$$

where

$$\begin{aligned} -b &= \lambda_1 + \lambda_2 = \alpha + \beta' + k_{+B}x_B + k_{-B} \\ c &= \lambda_1\lambda_2 = \alpha k_{-B} \left[1 + \frac{\beta'}{\alpha} \left(1 + \frac{x_B}{K_B} \right) \right] \end{aligned} \quad (31)$$

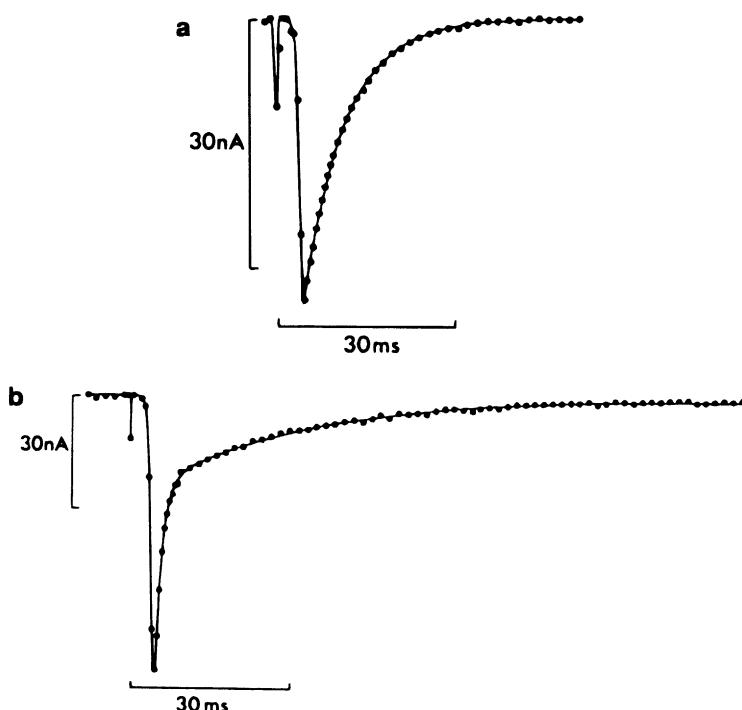
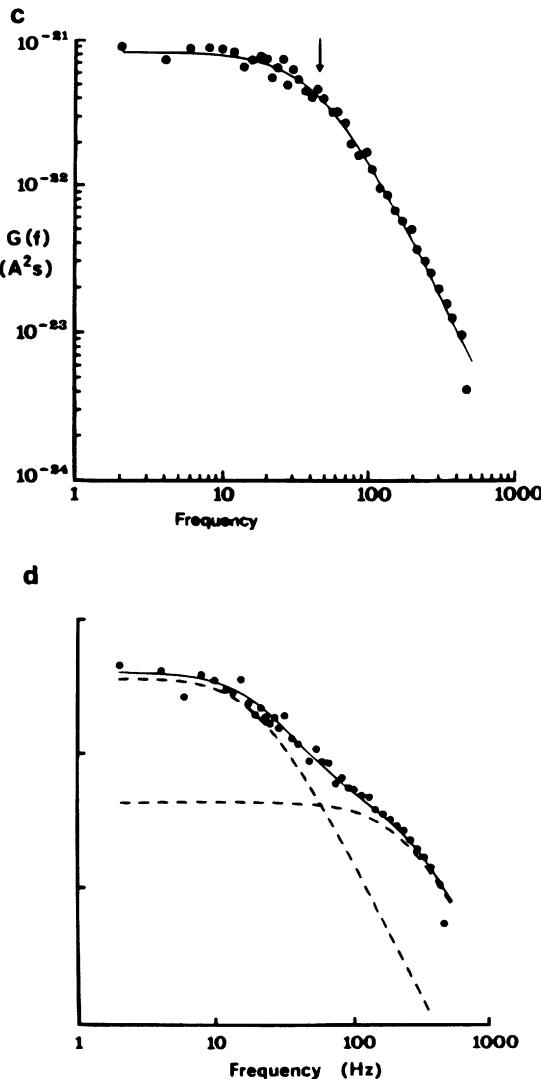


Figure 3. a and b: Endplate currents at -130 mV (dots) evoked by nerve stimulation (inward current is shown downward). a: Control, fitted with single exponential ($\tau = 7.1$ ms). b: In presence of $5 \mu\text{M}$ gallamine, fitted with sum of two exponentials ($\tau = 1.37$ and 28.1 ms). c and d: Spectral density (dots) of noise (at -100 mV) induced by carbachol. c: Carbachol ($20 \mu\text{M}$), fitted with single Lorentzian ($\tau = 3.47$ ms) d: Carbachol ($100 \mu\text{M}$) in presence of gallamine ($20 \mu\text{M}$), fitted with sum of two Lorentzians ($\tau = 0.65$ ms and 7.28 ms). Reproduced from Colquhoun and Sheridan (1981), with permission.

Figure 3. *Continued.*

and $K_B = k_{-B}/k_{+B}$ is the equilibrium constant for blocker binding. The relative amplitudes of the two components are also related to the reaction rate constants, though in a rather complicated way (see Colquhoun and Hawkes, 1977; Chapter 20, this volume).

4.3. Open Lifetimes of Single Channels

By contrast with noise or relaxation, the analysis of open times for single channels is very simple in this case. There is only one open state, and it is identifiable on the experimental record. By virtue of the rule given in expression 24, the open lifetime must therefore be

distributed exponentially with mean $1/(\alpha + k_{+B}x_B)$. This follows from expression 24 because there are two ways out of the open state (shutting or blocking) with transition rates α and $k_{+B}x_B$, respectively.

4.4. Shut Lifetimes of Single Channels

Because there are two indistinguishable shut states, this is not as simple as previous cases. However, in this particular mechanism, the two shut states cannot intercommunicate directly but only by going through the open state. This makes matters much simpler than they would otherwise be, because each period for which the channel is shut must consist *either* of a single sojourn in the shut state (exponentially distributed with mean $1/\beta'$) *or* of a single sojourn in the blocked state (exponentially distributed with mean $1/k_{-B}$). The overall distribution of shut times is therefore simply a mixture of these two distributions in proportions dictated by the relative frequency of sojourns in the shut and blocked states (as long as only one channel contributes to the observations; see Section 8). These frequencies will be proportional to α and $k_{+B}x_B$, respectively, because these rate constants give the relative frequencies (probabilities) with which each of the two shut states is entered, starting from the open state. Thus, the pdf of all shut times can be put into the general form of a mixture of (in this case) two exponentials (see equation 25), as

$$f(t) = a_1\beta'e^{-\beta't} + a_2k_{-B}e^{-k_{-B}t}$$

where the areas of the two components are

$$a_1 = \left(\frac{\alpha}{\alpha + k_{+B}x_B} \right) \quad \text{and} \quad a_2 = \left(\frac{k_{+B}x_B}{\alpha + k_{+B}x_B} \right) \quad (32)$$

4.5. Bursts of Openings

If the agonist concentration is low (β' is low), openings are infrequent, and if the blocker dissociates quite rapidly from the open channel (k_{-B} is large), blockages are brief. In this case, openings would be expected to occur in bursts as the channel blocks and unblocks several times in quick succession before entering a long shut period. This has been observed in many cases (e.g., Neher and Steinbach, 1978; Ogden *et al.*, 1981) and is illustrated in Fig. 4.

The burst-like appearance is just the single-channel equivalent of a double-exponential relaxation, as illustrated in Fig. 5 (compare Fig. 2, in which a simple exponentially distributed open lifetime gave rise to a simple exponential relaxation). But not all channel blockers will produce such obvious effects, as discussed next.

4.5.1. Fast Channel Blockers

Some low-affinity blockers produce very brief blockages (e.g., about 20 μ s for acetylcholine itself on nicotinic receptors; Ogden and Colquhoun, 1985). They therefore have noticeable effects only at high concentrations, at which blockages are very frequent. Bursts will consist

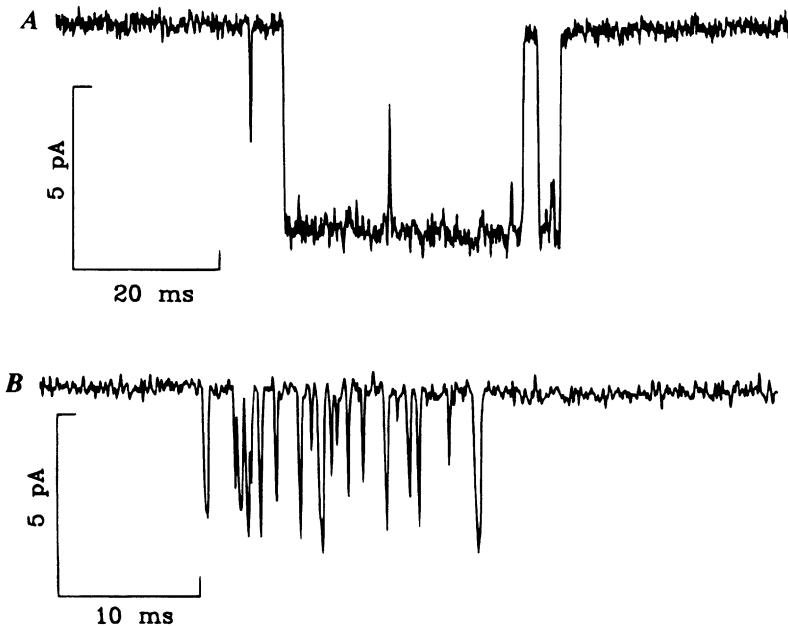


Figure 4. Channel blockage at the single-channel level, illustrated by the block of NMDA-type glutamate receptor channels by magnesium ions. The control trace in *A* shows openings of an NMDA receptor in the absence of magnesium (30 nM glutamate and 1 μ M glycine in calcium-free EDTA-buffered solution, with free Mg^{2+} about 0.2 μ M; see Gibb and Colquhoun, 1992). The trace in *B* was obtained in the presence of 25 μ M free Mg^{2+} (EDTA-buffered solution, 100 nM glutamate). Despite the complexity of the shut-time structure even in the absence of magnesium, the effect of channel blockage is very obvious. The channel openings in *B* are much shorter on average (so filtering prevents most of them from attaining full amplitude) and are frequently interrupted by brief blockages. Filtered at 3 kHz (-3 dB). (Unpublished results of A. J. Gibb on dissociated adult rat hippocampal CA1 cells.)

of a large number of very short openings ($k_{+B}x_B$ is large) separated by very short blockages (k_{-B} is large). Most of these are too brief to be clearly resolved, so the bursts look like single noisy openings of reduced amplitude. According to mechanism 29, we would expect to see only the slow component of the biphasic relaxation (see Fig. 5), which corresponds roughly to the burst length (see below). Such blockers would appear to slow down the relaxation. (In the case of very brief interruptions that occur with agonist alone, it is also true that the relaxation will be approximately exponential, with a time constant similar to the mean burst length; see Section 5 and Fig. 8.)

4.5.2. Slow Blockers

At the other extreme, some blockers produce very long blockages, e.g., tubocurarine on nicotinic receptors, with mean blockage durations of seconds (Colquhoun *et al.*, 1979). Such blockers will appear to speed up the relaxation. At the single-channel level individual openings will be shortened on average, as described below, but if, following the opening, there is a blockage that lasts for 3 s, it would be impossible to tell by looking at the record that the opening before the blockage and that after it were both part of one very long burst.

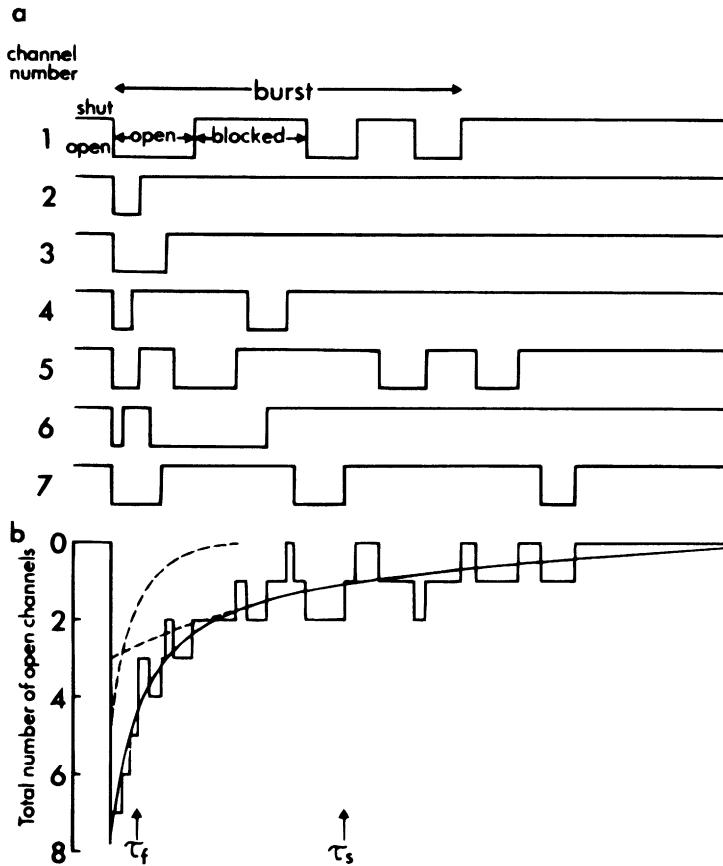


Figure 5. Schematic illustration to show why the occurrence of channel openings in bursts may result in biphasic relaxations (like, for example, that illustrated in Fig. 3b). The open state is shown as a downward deflection. a: Simulated behavior of seven individual ion channels in the presence of an ion-channel-blocking drug. Channels are supposed to be opened nearly synchronously at time zero by a quantum of acetylcholine, and the acetylcholine is supposed to disappear rapidly from the synaptic cleft. Thus, each channel produced only one burst of openings before it finally shuts (as marked on channel 1, which has two blockages and therefore three openings before it shuts). b: Sum of all seven records shown in a. The initial decline is rapid (time constant τ_f) as open channels become blocked, but the current thereafter declines more slowly (time constant τ_s). The continuous line is the sum of two exponential curves (shown separately as dashed lines) with time constants τ_f and τ_s . The slow time constant, under these conditions, reflects primarily the burst length rather than the length of an individual opening. (See also Neher and Steinbach, 1978.)

At the macroscopic level, the relaxation would reflect only the shortened openings; there would in fact be a very slow component too, but it would have such small amplitude that it would be undetectable.

4.6. The Number of Openings per Burst

The number of openings per burst will, of course, be a random variable. Its distribution can be found as follows. Define as π_{12} the probability that an open channel (state 1) will,

as its next transition, become blocked (state 2). This probability takes no account of how much time elapses before the transition occurs but only of where the transition leads when it eventually does occur. It therefore depends simply on the rate of transition from state 1 to state 2, $k_{+B}x_B$; this rate must be divided by the sum of all rates for leaving state 1 so that the probabilities add to unity. Thus, we obtain

$$\pi_{12} = \frac{k_{+B}x_B}{\alpha + k_{+B}x_B} \quad (33)$$

which is precisely what was used in equation 32 to define the relative frequency of entry into each shut state. If the open channel does not block next, the only other possibility is that it shuts next, so the probability that the next transition of the open channel is to the shut state (state 3) is

$$\pi_{13} = 1 - \pi_{12} = \frac{\alpha}{\alpha + k_{+B}x_B} \quad (34)$$

We shall also need the probability that the next transition of the blocked channel is to the open state. In this particular mechanism, equation 29, there is nowhere else the blocked channel can go, so

$$\pi_{21} = 1 \quad (35)$$

The probability that a burst has only one opening is simply the probability that the channel, once open, then shuts, i.e., π_{13} . If the burst has two openings (and therefore one blockage), the open channel first blocks (probability π_{12}), then reopens (probability π_{21}), and finally shuts (probability π_{13}). So the overall probability of seeing two openings is the product of these three probabilities, i.e., $(\pi_{12}\pi_{21})\pi_{13}$. Extension of this argument gives the probability of a burst having r openings (and $r - 1$ blockages) as

$$P(r) = (\pi_{12}\pi_{21})^{r-1}\pi_{13} = (\pi_{12}\pi_{21})^{r-1}(1 - \pi_{12}). \quad (r = 1, 2, \dots, \infty) \quad (36)$$

This form of distribution is called a *geometric distribution*. The cumulative form of this distribution, the probability that we observe n or more openings per burst, is

$$P(r \geq n) = (\pi_{12}\pi_{21})^{n-1} \quad (37)$$

The mean number of openings per burst (denoted m_r) is

$$m_r = \sum_{r=1}^{\infty} rP(r) = \frac{1}{1 - \pi_{12}} = 1 + \frac{k_{+B}x_B}{\alpha} \quad (38)$$

This last result predicts that the mean number of openings per burst should increase linearly with the blocker concentration, with slope k_{+B}/α .

The number of blockages per unit open time is predicted to be simply $k_{+B}x_B$, so a plot

of the observed blockage frequency against x_B should go through the origin and have a slope of k_{+B} . This behaviour has been observed directly in some cases, as illustrated in Fig. 6.

The geometric distribution is the discrete equivalent of the exponential distribution. It has the characteristic that a given increment in r reduces $P(r)$ by a constant factor (viz. $\pi_{12}\pi_{21}$), which is analogous to the behaviour of an exponential. And when m_r is large, the geometric distribution is well approximated by an exponential distribution with mean m_r . More generally, we expect that, under conditions where distributions of time intervals are described by a mixture of exponentials (see equation 25), the distributions of quantities such as the number of openings per burst (which can adopt only discrete integer values) will be described by a mixture of geometric distributions (see also Chapter 19, this volume). The number of geometric components in the distribution of the number of openings per burst is determined by the number of routes between open states and short-lived shut states (see Section 13.4) and is therefore not more than the number of open states (see also Chapter 20, this volume; Colquhoun and Hawkes, 1982, 1987).

4.7. Lifetime of Various States and Compound States

From the rule obtained earlier, in equation 24, we can immediately obtain the distribution of lifetimes in the three individual states. These will be exponentially distributed with

$$\text{Mean open lifetime: } m_0 = 1/(\alpha + k_{+B}x_B) \quad (39)$$

$$\text{Mean blocked lifetime (gap within a burst): } m_w = 1/k_{-B} \quad (40)$$

$$\text{Mean shut lifetime (gap between bursts): } m_b = 1/\beta' \quad (41)$$

Thus, if bursts can clearly be distinguished in the observed record, we can, for example, obtain an estimate of k_{-B} simply by measuring the mean length of gaps within bursts. The

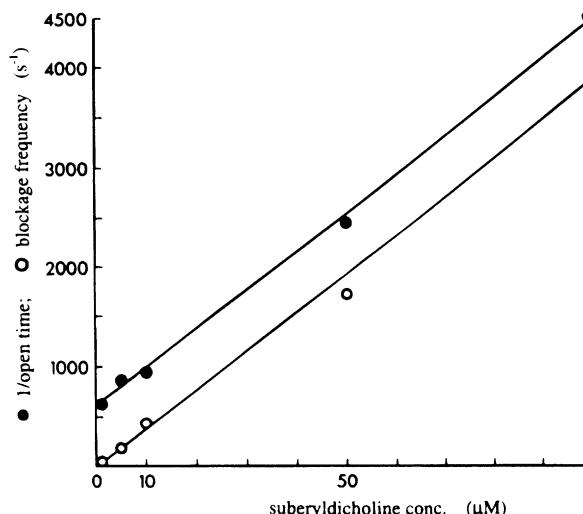


Figure 6. Block of suberyldicholine-activated endplate channels by the agonist itself at high concentrations. The blockages produce a characteristic component with a mean of about 5 ms in the distribution of shut times (so $k_{-B} \approx 1/5$ ms = 200 s⁻¹). Blockages become more frequent (the relative area of the 5-ms component increases) with concentration. *Closed circles:* reciprocal of the corrected mean open time, plotted against concentration (slope 3.9×10^7 M⁻¹s⁻¹ intercept 594 s⁻¹). *Open circles:* the ‘blockage frequency plot’—the frequency of blockages per unit of open time, plotted against concentration (slope = 3.8×10^7 M⁻¹s⁻¹, intercept 4 s⁻¹). Reproduced with permission from Ogden and Colquhoun (1985).

division of openings into bursts may be a particularly useful procedure if more than one channel is contributing to the experimental record (see Section 8). In this case, it will not be known whether a burst originates from the same channel as the previous burst, so the lengths of shut times (gaps) *between* bursts will not be interpretable. Usually, however, it will be likely that all openings within a particular burst originate from the same channel, so the lengths of gaps within bursts will be interpretable and useful.

By use of the means in equations 39–41, we can immediately obtain the average value for the total open time during a whole burst. It will be the mean number of openings per burst, m_r , from equation 38, multiplied by the mean length of an opening, m_0 from equation 39. Thus,

$$\begin{aligned}\text{Mean open time per burst} &= m_r m_0 = (1 + k_{+B} x_B / \alpha) \left(\frac{1}{\alpha + k_{+B} x_B} \right) \\ &= 1/\alpha\end{aligned}\quad (42)$$

Thus, the mean open time per burst is exactly what the mean length of an opening would have been if no blocker were present, as was first pointed out by Neher and Steinbach (1978). This result seems surprising at first, and it will be discussed again in Section 6. Similarly, the total length of time spent in the blocked state per burst (total shut time per burst) is, on average,

$$\text{Mean shut time per burst} = (m_r - 1)m_w = \frac{k_{+B} x_B}{\alpha} (1/k_{-B}) = c_B / \alpha \quad (43)$$

where we denote the blocker concentration, normalized with respect to its equilibrium constant ($K_B = k_{-B}/k_{+B}$), as

$$c_B = x_B / K_B \quad (44)$$

Addition of equations 42 and 43 gives the mean burst length as

$$\text{Mean burst length} = \frac{1 + c_B}{\alpha} \quad (45)$$

as derived by Neher and Steinbach (1978). This is predicted to increase linearly with the concentration of blocker.

We have just obtained means for the durations of various quantities characteristic of the burst, but so far we have not mentioned the distribution of these variables. It can be shown (see below; Colquhoun and Hawkes, 1982) that the fact that there is only one open state implies that the total open time per burst has a simple exponential distribution (with mean $1/\alpha$ as found above). Similarly, the fact that the gaps within bursts are spent in a single state (state 2, the blocked state) implies that the total shut time per burst (excluding bursts that have no blockages in them) will also have a simple exponential distribution, with the overall mean derived in equation 43 divided by the probability that there is at least one blockage, which, from equation 37, is $P(r \geq 2) = \pi_{12}\pi_{21}$.

The distribution of the number of openings per burst had one (geometric) component because there is only one open state in this example. However, the distribution of the burst

length will be described by the sum of two exponential terms (because the burst is a period of time spent in either of two states, open or blocked). This distribution can be derived as follows.

4.8. Derivation of Burst Length Distribution for the Channel-Block Mechanism

We note that a burst consists of a sojourn in either of two states, open or blocked. As soon as the shut state (see equation 29) is entered, the burst ends. Thus, we want to find the distribution of the time spent oscillating within the pair of burst states (open \rightleftharpoons blocked) without leaving this pair for the shut state.

We have already considered one example, the distribution of all shut times, that involved a sojourn in a pair of states, but this was unusually simple to deal with because the two shut states in question (shut and blocked) could not intercommunicate. In this case, the two states of interest (open and blocked) can intercommunicate, so a more general approach is needed; a similar approach can be used for many problems that involve a sojourn in a set of two or more states.

The burst starts at the beginning of the first opening and ends at the end of the last opening; the channel is open at the start and end of the burst. We have already considered in equation 8 a probability defined as

$$P_{11}(t) = \text{Prob}(\text{open at time } t | \text{open at time } 0) \quad (46)$$

This is what is needed for derivation of the time course of the macroscopic current or for the noise spectrum. However, it is not quite what we need now; this probability allows for the possibility that the system may enter any of the other states between 0 and t , but if the shut state is entered, the burst is ended, and we are no longer interested. What we need is a modified version of this that restricts the system to staying in the burst (i.e., in either open or blocked states) *throughout* the time between 0 and t . This sort of probability will be denoted by a prime. Thus,

$$P'_{11}(t) = \text{Prob}(\text{stays in burst throughout } 0, t \text{ and open at } t | \text{open at } 0) \quad (47)$$

By analogy with the procedure in Section 3, we start by obtaining an expression for $P'_{11}(t + \Delta t)$, the probability that the channel stays within a burst for the whole time from 0 to $t + \Delta t$ and is open at $t + \Delta t$, given that it was open at $t = 0$. This can happen in either of two ways (the probabilities of which must be added):

1. The channel is open at t [with probability $P'_{11}(t)$] *and* stays open during the interval Δt between t and $t + \Delta t$. The probability that the channel does *not* stay open during Δt is $(\alpha + k_{+B}x_B)\Delta t + o(\Delta t)$, so the probability that it does stay open during Δt is $1 - (\alpha + k_{+B}x_B)\Delta t - o(\Delta t)$.
2. The channel is blocked (state 2) at time t [the probability of this, following the notation in equation 8 is $P'_{12}(t)$], *and* the channel unblocks during Δt [with probability $k_{-B}\Delta t + o(\Delta t)$]. Assembling these values gives our required result as

$$P'_{11}(t + \Delta t) = P'_{11}(t)[1 - (\alpha + k_{+B}x_B)\Delta t - o(\Delta t)] + P'_{12}(t)[k_{-B}\Delta t + o(\Delta t)] \quad (48)$$

Rearrangement of this, followed by allowing Δt to tend to zero, gives (by the method used in equations 17 and 18)

$$\frac{dP'_{11}(t)}{dt} = -(\alpha + k_{+B}x_B)P'_{11}(t) + k_{-B}P'_{12}(t) \quad (49)$$

This cannot be solved as it stands because there are two unknowns, $P'_{11}(t)$ and $P'_{12}(t)$. However, if an exactly analogous argument to that just given for $P'_{11}(t)$ is followed for $P'_{12}(t)$, we obtain another differential equation,

$$\frac{dP'_{12}(t)}{dt} = k_{+B}x_B P'_{11}(t) - k_{-B} P'_{12}(t) \quad (50)$$

We now have two simultaneous equations in two unknowns, which can be solved. For example, equation 49 can be rearranged to give an expression for $P'_{12}(t)$, which is substituted into equation 50. In this way, $P'_{12}(t)$ is eliminated, and we obtain a single (second-order) equation in $P'_{11}(t)$ only:

$$\frac{d^2P'_{11}(t)}{dt^2} + \frac{dP'_{11}(t)}{dt} (\alpha + k_{+B}x_B + k_{-B}) + \alpha k_{-B} P'_{11}(t) = 0 \quad (51)$$

Standard methods give the solution of this as the sum of two exponential terms with rate constants λ_1 and λ_2 :

$$P'_{11}(t) = \frac{1}{\lambda_2 - \lambda_1} [(k_{-B} - \lambda_1)\exp(-\lambda_1 t) + (\lambda_2 - k_{-B})\exp(-\lambda_2 t)] \quad (52)$$

The two rate constants, λ_1 and λ_2 , are found by solution of the quadratic equation,

$$\lambda^2 + b\lambda + c = 0$$

where

$$\begin{aligned} -b &= \lambda_1 + \lambda_2 = \alpha + k_{+B}x_B + k_{-B} \\ c &= \lambda_1\lambda_2 = \alpha k_{-B} \end{aligned} \quad (53)$$

The pdf for the burst length follows directly from this. It is defined as

$$f(t) = \lim_{\Delta t \rightarrow 0} [\text{Prob(burst lasts from } 0 \text{ to } t \text{ and leaves burst in } t, t + \Delta t)/\Delta t] \quad (54)$$

In this case, the burst can be left only by direct transition from the open state to the shut state; the blocked state cannot shut directly, so there is no possibility of an experimentally invisible period in the blocked state following the last opening (cf. the example in Section 5). The probability that the burst is left in $t, t + \Delta t$ is simply $\alpha\Delta t + o(\Delta t)$, conditional on the burst lasting from 0 to t and being in open state 1 at t . Insertion of this into equation 54 gives

$$f(t) = \lim_{\Delta t \rightarrow 0} \{P'_{11}(t)[\alpha\Delta t + o(\Delta t)]/\Delta t\} = \alpha P'_{11}(t) \quad (55)$$

where $P'_{11}(t)$ is given by equation 52. Thus, the final form of the distribution of the burst length is, in the standard form specified in equation 25,

$$f(t) = a_1 \lambda_1 e^{-\lambda_1 t} + a_2 \lambda_2 e^{-\lambda_2 t} \quad (56)$$

where the areas of the two components are

$$a_1 = \frac{\alpha(k_B - \lambda_1)}{\lambda_1(\lambda_2 - \lambda_1)} \quad \text{and} \quad a_2 = \frac{\alpha(\lambda_2 - k_B)}{\lambda_2(\lambda_2 - \lambda_1)} \quad (57)$$

The mean burst length follows from

$$m = \int_0^\infty t f(t) dt = \frac{a_1}{\lambda_1} + \frac{a_2}{\lambda_2} = \frac{(1 + c_B)}{\alpha} \quad (58)$$

which agrees with the result already found in equation 45 by a different route.

Two things are noteworthy about this distribution. (1) Unlike the simple case in which states do not intercommunicate, which was exemplified in equation 32, the two rate constants defined by equation 53 are compound quantities with no exact physical significance. (2) The two rate constants found here are not the same as those found for noise and relaxation experiments, as given in equations 30 and 31. The present versions are simpler because they do not involve rate constants that are concerned only with transitions from states outside the burst; i.e., they do not involve β' in this case. However, if few channels are open (β' is small), the rate constants for noise and relaxation, from equation 31, will become similar to those for the burst length distribution, from equation 53.

5. A Simple Agonist Mechanism

The mechanism of Castillo and Katz (1957), which has already been discussed in Sections 1.1 and 3.5, also has, like that just discussed, two shut states and one open state. However, in this case, the two shut states can intercommunicate directly. It will be convenient to number the three states thus



State Number	3	2	1
--------------	---	---	---

5.1. Shut Times

This mechanism can be analyzed in much the same way as the channel-block mechanism. In this case, because the two shut states intercommunicate, the distribution of all shut periods,

although it will still have two exponential terms, will not have rate constants that have a simple physical significance. The rate constants must be found by solving a quadratic as in equation 53. The derivation follows the same lines as that for the distribution of the burst length and is given in full by Colquhoun and Hawkes (1994), so it will not be repeated here.

5.2. Bursts of Openings

Again, openings are predicted to occur in bursts, in this case bursts of several openings during a single occupancy (i.e., oscillation between AR and AR* before final dissociation). The bursts will be obvious as long as the time spent in AR, on average $1/(\beta + k_{-1})$ from rule 24, is short compared with the time between bursts. The distribution of the gap between bursts will be complicated by the fact that repeated occupancies ($R \rightleftharpoons AR$) may take place before a burst starts, and the gap between bursts will also include the time spent in AR immediately before the first opening of the burst and immediately after the last opening, as illustrated in Fig. 7.

The distribution of the number of openings per burst is geometric (as in Section 4.6), with mean, m_r , given by

$$m_r = 1 + (\beta/k_{-1}) \quad (60)$$

The openings have mean length $1/\alpha$, so the mean open time per burst is therefore m_r/α . Each burst will contain, on average, $(m_r - 1)$ brief shuttings, each of mean length $1/(\beta + k_{-1})$, giving a mean total shut time per burst of $(m_r - 1)/(\beta + k_{-1})$. The mean burst length will be the sum of these two quantities.

The distribution of the burst length can be found, much as in the channel-block example (Section 4.8), by deriving an expression for $P'_{11}(t)$. The way that the burst ends is rather different in this case, however; it cannot end (reach state 3) directly from the open state but only via AR. Therefore $P'_{11}(t)$ must be multiplied not only by the transition rate from AR* to AR, i.e., by α , as in equation 55, but also by the probability that, once in AR, the burst ends rather than continues, i.e., $\pi_{23} = k_{-1}/(\beta + k_{-1})$.

The openings of many sorts of ion channel are observed to occur in bursts, as illustrated

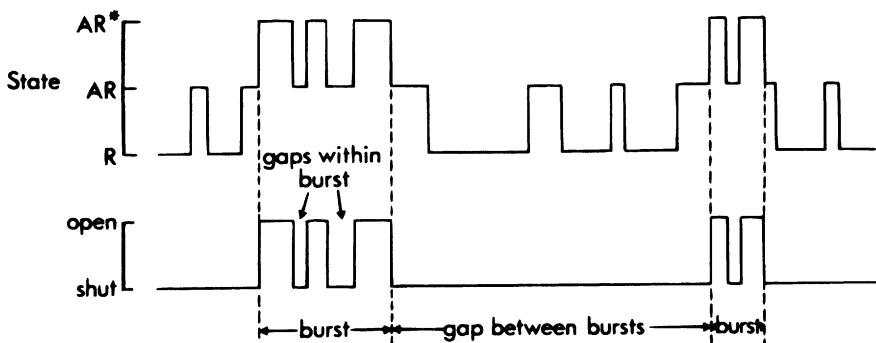


Figure 7. Schematic illustration of transitions between various states (top) and observed single-channel currents (bottom) for the simple agonist mechanism in equation 59. This illustrates the molecular events that underlie a burst of openings.

in Fig. 8 for the nicotinic acetylcholine receptor. Such observations have been interpreted along the lines suggested above, though a somewhat more complex mechanism than 59 is needed, as discussed in Sections 11 and 13; Chapter 20, this volume; Colquhoun and Hawkes, 1994; see, for example, Colquhoun and Sakmann, 1985; Sine and Steinbach, 1986).

5.3. Effective Openings

If the resolution of the experiment is poor, few of the brief shuttings, of the sort shown in Fig. 8 will be detected, and the bursts will appear to be single openings (see Section 12), with mean length equal to the burst length. When the shut times within bursts are short, the mean length of this “effective opening” will be little different from the total open time per burst, m_r/α , i.e., from equation 60,

$$\text{Mean open time per burst} = \frac{1}{\alpha} \left(1 + \frac{\beta}{k_{-1}} \right) \quad (61)$$

Furthermore, by virtue of equation 28, the duration of the “effective opening” will be approximately a single exponential with this mean.

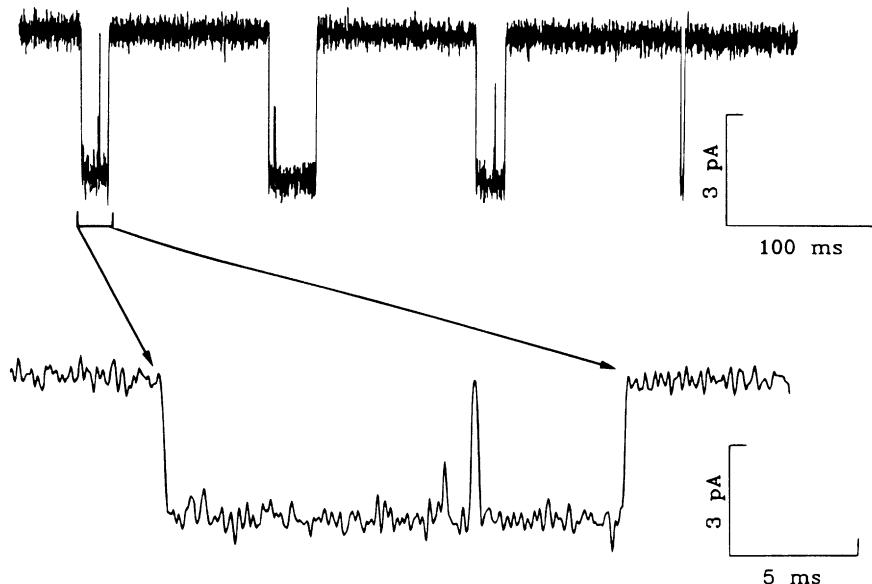


Figure 8. Example of bursts of channel openings elicited by an agonist. The upper trace shows four bursts of openings elicited by acetylcholine (100 nM, adult frog endplate, filter 2.5 kHz –3 dB; unpublished data of D. Colquhoun and B. Sakmann, methods as in Colquhoun and Sakmann, 1985). The last burst appears to consist of a single short opening, but the other three contain at least two or three openings separated by short shut periods. The lower section shows the first burst on an expanded time scale. It contains one fully resolved shut period and a partially resolved shutting. On the assumption that the partially resolved event is indeed a complete closure, time-course fitting (see Chapter 19, this volume) suggests that the burst contains three openings (durations 10.7 ms, 1.0 ms and 5.7 ms) separated by two closed periods (durations 61 µs and 289 µs).

If a channel-blocking agent is added to the mechanism in equation 59, there will now be three shut states rather than two. If the channel blockages are, on average, much longer than the spontaneous brief shuttings just discussed, then each activation of the channel can be considered as a *cluster* of openings, the bursts within a cluster being separated by channel blockages, and the openings within a burst being separated by spontaneous brief shuttings. The formal theory of clusters of bursts was presented by Colquhoun and Hawkes (1982). This theory was used by Ogden and Colquhoun (1985) to show that, in the case where the spontaneous brief shuttings cannot be resolved, the whole burst will behave approximately like a single “effective opening,” and application of the simple channel-block theory given in Section 4 will not give rise to serious errors. For example, the mean length of the effective opening will be reduced by the presence of a channel blocker in the same way as the mean length of the actual openings is reduced.

5.4. Macroscopic Currents

When the gaps within bursts are brief, noise and relaxation experiments will give a time constant that corresponds approximately to the mean burst length (rather than the mean open time). This can be shown as follows.

The two macroscopic rate constants are found, as usual, by solving a quadratic equation,

$$\lambda^2 + b\lambda + c = 0.$$

The well-known solution of this quadratic is

$$\lambda_1, \lambda_2 = 0.5(-b \pm \sqrt{b^2 - 4c}) \quad (62)$$

A less well-known alternative is

$$\lambda_1, \lambda_2 = \frac{2c}{-b \mp \sqrt{b^2 - 4c}} \quad (63)$$

where, as before,

$$-b = \lambda_1 + \lambda_2, \quad c = \lambda_1 \lambda_2 \quad (64)$$

When one of the rate constants is much bigger than the other (say $\lambda_f \gg \lambda_s$, where the subscripts denote *fast* and *slow*), i.e., when $b^2 \gg c$, then the faster rate constant, λ_f , is approximately

$$\lambda_f \approx -b \quad (65)$$

and, from the alternative form, equation 63, the slower rate constant, λ_s , is approximately

$$\lambda_s \approx -c/b \quad (66)$$

In this case the coefficients are given (e.g., Colquhoun and Hawkes, 1977) by

$$-b = \lambda_s + \lambda_f = \alpha + \beta + k_{+1}x_A + k_{-1} \quad (67)$$

$$\begin{aligned}
 c &= \lambda_s \lambda_f = \alpha k_{-1} \left[1 + \frac{x_A}{K_A} \frac{(\alpha + \beta)}{\alpha} \right] \\
 &= \alpha k_{-1} + \alpha k_{+1} x_A + \beta k_{+1} x_A
 \end{aligned} \tag{68}$$

where K_A is the microscopic equilibrium constant for the binding reaction, i.e., k_{-1}/k_{+1} . When the shut times within bursts are very short, only the slower of these components will be detectable, and the time constant for this component will, from equation 66, be approximately

$$\begin{aligned}
 \tau_s = 1/\lambda_s &\approx \frac{\alpha + \beta + k_{+1}x_A + k_{-1}}{\alpha k_{-1} + \alpha k_{+1}x_A + \beta k_{+1}x_A} \\
 &\approx \frac{1}{\alpha} \left(1 + \frac{\beta}{k_{-1}} \right)
 \end{aligned} \tag{69}$$

The second approximation is valid (1) when the agonist concentration, x_A , is sufficiently low, and (2) when the shut times within bursts, with mean length $1/(\beta + k_{-1})$, are short enough that $(\beta + k_{-1}) \gg \alpha$. The result in equation 69 is seen to be the mean open time per burst, as found in equation 61, i.e., approximately the mean burst length. Several approximations had to be made to get this result; this illustrates the general principle that there is usually no simple correspondence between the time constants for macroscopic currents and the time constants for the single-channel distributions.

This topic is discussed further in Sections 11 and 13 (see also Chapter 20, this volume; Colquhoun and Hawkes, 1981, 1982, 1994).

6. Some Fallacies and Paradoxes

The random nature of single-channel events leads to behaviour that is often not what might, at first sight, be expected intuitively. Some examples of apparently paradoxical behaviour and of common fallacies are now discussed.

6.1. The Waiting Time Paradox

This is most easily illustrated by consideration of a simple binding reaction



Imagine that the receptors (R) have attained equilibrium with a concentration x_A of the ligand (A). The fraction of receptors that are occupied (or the fraction of time for which a particular receptor is occupied) will be $p_1(\infty) = x_A/(x_A + K_A)$, where $K_A = k_{-1}/k_{+1}$. Suppose that at an arbitrary moment, $t = 0$, the ligand concentration is reduced to zero. We should expect

to see a simple exponential decline in receptor occupancy with a time constant $1/k_{-1}$ (though in practice diffusion problems usually preclude such simplicity). This, of course, would be interpreted (see Fig. 2) in stochastic terms by pointing out that the length of time for which a particular receptor remains occupied is exponentially distributed with a mean of $1/k_{-1}$, from rule 24.

However, it might be objected that a receptor that is occupied at the arbitrary moment $t = 0$ must already have been occupied for some time before $t = 0$, and what we measure in the experiment is the residual lifetime of the occupied state from $t = 0$ until dissociation eventually occurs, as illustrated in Fig. 9. Because the mean lifetime of the entire occupancy, measured from the moment the receptor becomes occupied to the moment of dissociation, is on average $1/k_{-1}$, surely this residual lifetime should be shorter! On the other hand, since the drug-receptor complex does not 'age'—i.e., it has no knowledge of how long it has already existed—the mean lifetime measured from any arbitrary moment must always be $1/k_{-1}$. Both of these arguments sound quite convincing, but the latter argument is the correct one.

The resolution of the paradox lies in the fact that we are looking, in the experiment, only at those drug-receptor complexes that happened to exist at the moment, $t = 0$, when we chose suddenly to reduce the ligand concentration to zero (no more complexes can form after this moment). These particular complexes will not be typical of all drug-receptor complexes: we have a greater chance of catching in existence long-lived complexes than short-lived ones. This happens because of a phenomenon known as length-biased sampling. Although complexes with above-average lifetimes are *fewer* in number than those with below-average lifetimes (because of the positive skew of the exponential distribution), the former actually occupy a *greater* proportion of the total time than the latter. The above-average lifetimes have, therefore, a greater probability of being caught in existence at an arbitrary moment. Although the mean length of all occupancies is $1/k_{-1}$, the mean lifetime of the particular complexes that are in existence at $t = 0$ is twice as long, $2/k_{-1}$. These complexes will, on average, have been in existence for a time $1/k_{-1}$ before $t = 0$ and for a time $1/k_{-1}$ (the residual lifetime) after $t = 0$. The paradox is resolved. Further details can be found, for example, in Feller (1966) or Colquhoun (1971, Chapter 5 and Appendix 2).

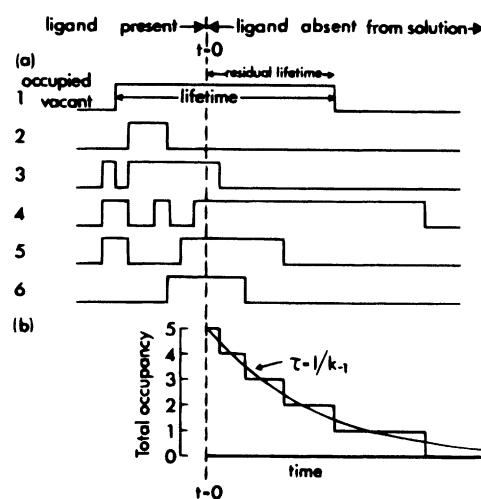


Figure 9. Illustration of the waiting time paradox. (a) Simulated behavior of six individual receptors. Before $t = 0$, ligand is present, and the receptor becomes occupied and vacant at random. The average lifetime of an occupancy is $1/k_{-1}$ (where k_{-1} is the dissociation rate constant). At $t = 0$, the ligand is removed from solution, and receptors that were occupied at $t = 0$ dissociate after a variable length of time. (b) The total of the records in a, showing the time course of decline of occupancy. The time course clearly reflects the distribution of the residual lifetime (defined on channel 1), which turns out to be identical with the distribution of the total lifetime (also defined on channel 1). Both are exponentially distributed with mean $1/k_{-1}$.

6.2. The Unblocked Channel Fallacy

Consider the simple channel-block mechanism of equation 29. In the absence of the blocking drug, the mean length of an opening would be $1/\alpha$. It was found above that in the presence of the blocker in concentration x_B , the mean length of an individual opening is reduced to $1/(\alpha + k_{+B}x_B)$. The easiest way to imagine why the opening is, on average, shorter is to suppose that its normal lifetime is cut short by a blocking molecule, which causes it to cease conducting prematurely (before it would otherwise have shut). But not every opening is ended by a blockage. The number of blockages per burst is a random variable, and a certain number of openings will end in the normal way, by transition to the shut state, rather than by the channel being blocked. This will be true of openings that have no blockage, so there is only one opening in the burst (and, more generally, for the last opening in any burst). Surely, these openings, which have not been cut short by a blockage, must be perfectly normal, with a mean lifetime $1/\alpha$.

On this basis, it is sometimes suggested, for example, that the noise spectrum should contain a component with the normal time constant ($1/\alpha$), which corresponds to those channels that do not block. However, this is quite inconsistent with rule 24, which states that because there is only one open state, its lifetime must follow a simple exponential distribution with a mean, in this example, of $1/(\alpha + k_{+B}x_B)$. There should be no component with mean $1/\alpha$. In fact, if openings that end by shutting in the normal way rather than by blocking (e.g., bursts with only one opening) were measured separately from all other openings, it would be found that their duration was a simple exponential with mean $1/(\alpha + k_{+B}x_B)$; they *are* shorter than “normal” even though no blockage has occurred. The reason is again connected with length-biased sampling. Openings that happen to be very long will tend to get blocked before they shut, so, conversely, the openings that happen to be short (less than $1/\alpha$) will predominate among those that have no blockage. The extent to which these are shorter than $1/\alpha$ turns out, with great elegance, to be precisely sufficient to make their mean lifetime $1/(\alpha + k_{+B}x_B)$, exactly the same as that for openings that *are* terminated by being blocked.

6.3. The Last Opening of a Burst Fallacy

There are a number of other fallacies that can be disposed of easily by rule 24, which gives the distribution of the length of time spent in a single state. The explanation is, as in the last example, usually based on length-biased sampling. For example, the simple agonist mechanism, equation 59 predicts that openings should occur in bursts. The average length of an opening should be $1/\alpha$ regardless of where it occurs in the burst as long as there is only one open state (though if there is more than one open state, this may no longer be true; see below). According to mechanism 59, the agonist cannot dissociate from the open channel. If it were able to, it might be thought that this dissociation would end the burst and would cut short the lifetime of the last opening in the burst. Thus, might it be possible to test the hypothesis that the agonist can dissociate from the open state by seeing whether the last opening of the burst has a different distribution from the others? If there is only one open state, clearly this would *not* be possible. It is true that if a channel could shut by another route as well as that shown in equation 59, the mean lifetime of the open state would be reduced to something less than $1/\alpha$. But all openings regardless of position in the burst would have, on average, this same reduced lifetime.

6.4. The Total Open Time per Burst Paradox

It was pointed out earlier, in equation 42, that for the simple channel-block mechanism, the total time per burst that is spent in the open state will be, on average, $1/\alpha$. This is exactly what the mean open time would be in the absence of a blocker (a fact that, incidentally, explains the inefficiency of channel block in reducing the equilibrium current when the agonist concentration is low). How can this happen? The channel cannot ‘know’ how long it has been open earlier in the burst and so make up the total open time to $1/\alpha$. After a blockage, the channel is not continuing a normal open time (mean length $1/\alpha$) but starting a new open time [with mean length $1/(\alpha + k_{+B}x_B)$]. Clearly, since the mean length of a single opening is $1/(\alpha + k_{+B}x_B)$, it follows at once that the mean open time per burst for bursts with r openings must simply be $r/(\alpha + k_{+B}x_B)$. The relative proportions of bursts with $r = 1, 2, \dots$ openings, given by equation 36, must be such that, on average, the total open time per burst is $1/\alpha$.

One way of understanding this is as follows (see Colquhoun and Hawkes, 1982). Imagine that a clock is started at the beginning of the first opening of a burst; the clock is stopped when the channel blocks and restarted when the channel reopens. It is finally stopped at the end of the burst, i.e., as soon as the channel shuts (as opposed to blocking). Thus, the clock runs only while the channel is open, and when finally stopped, it shows the total open time per burst. While the channel is open, the probability that it will leave the open state in Δt is $(\alpha + k_{+B}x_B)\Delta t + o(\Delta t)$, but if it leaves for the blocked state, the clock is stopped only temporarily. For the whole time that the clock is running, the probability that the clock is stopped finally, i.e., that the channel shuts (as opposed to blocking), in Δt is $\alpha\Delta t + o(\Delta t)$. This fact is sufficient to ensure that the time shown when the clock stops finally, the total open time per burst, has a simple exponential distribution with mean $1/\alpha$; this follows from the derivation of the exponential distribution given in Section 3.

A more general treatment (see Section 13; Chapter 20, this volume; Colquhoun and Hawkes, 1982; Neher, 1983) shows that the total open time per burst will be $1/\alpha$ for any mechanism with one open state as long as it fulfills the following condition. Suppose that there are any number of short-lived shut states (\mathcal{B} states, say) in which the system stays during a gap within a burst, and that there are any number of long-lived shut states (\mathcal{C} states, say) in which the system stays during a gap between bursts. If the only route from the former set of states (\mathcal{B}) to the latter (\mathcal{C}) is via the open state, and the total transition rate from the open state to the \mathcal{C} states is α , then the total open time per burst must be, on average, $1/\alpha$. If, on the other hand, there are routes from \mathcal{B} states to \mathcal{C} states that do not go through the open state (e.g., if the blocked channel can shut without reopening in the channel block example), the mean open time per burst must be less than $1/\alpha$.

7. Reversible and Irreversible Mechanisms

Most reaction mechanisms are such that the system, left to itself, will move spontaneously towards a true thermodynamic equilibrium. All the reaction steps in such mechanisms will usually be reversible, and the mechanism must obey the principle of microscopic reversibility or detailed balance (see Denbigh, 1951). This principle states that at equilibrium, each individual reaction step will proceed, on average, at the same rate in each direction. This means, for example, that a cyclic reaction mechanism cannot have, at equilibrium, any tendency to move predominantly in one direction around the cycle; this has implications for

the form of the distribution of open times (see example below). A slightly more subtle consequence of the principle of microscopic reversibility is that it implies that the stochastic properties of the mechanism must show time reversibility; they must be, on average, the same whether the record is read from left to right or from right to left (see Kelly, 1979). One example of such time symmetry is given by Colquhoun and Hawkes (1982), who discuss a mechanism in which all openings in a burst have not got the same distribution; these distributions are, however, the same for the first and last openings, and for the second and next-to-last openings, and so on. Another example is discussed below.

Reaction mechanisms with irreversible steps, such as that in equation 74 below, do not obey the principle of microscopic reversibility and do not tend spontaneously towards equilibrium. Such reactions may, however, be maintained in a steady state if they are coupled to a source of energy. If a steady state is attained, then all of the distributions derived by Colquhoun and Hawkes (1982) are still valid, although time symmetry is not, of course, expected.

7.1. A Simple Example

Some of the consequences of reversibility and irreversibility can be illustrated by a simple example, a cyclic mechanism that has one shut state (C) and two open states (O_1 and O_2). First consider the possibility that there might be a net clockwise circulation around the cycle. To achieve this, we might assign rate constants (all with dimensions s^{-1}) as follows:



For reversible reactions, however, the principle of microscopic reversibility implies that the product of the rate constants going one way around the cycle is the same as the product going the other way around. The rate constant for $O_2 \rightarrow O_1$, which has been omitted from scheme 70, must therefore be $2450 s^{-1}$. The complete mechanism is thus



Denote the closed state as state 3 and the open states O_1 and O_2 as states 1 and 2, respectively. At equilibrium, the occupancy of each state is

$$\begin{aligned}
 p_1(\infty) &= 0.4851 \\
 p_2(\infty) &= 0.0198 \\
 p_3(\infty) &= 0.4951
 \end{aligned} \tag{72}$$

Thus, the mean frequency of the $O_1 \rightarrow O_2$ transitions, $0.4851 \times 100 = 48.51$ per second, is the same as the mean frequency of the $O_2 \rightarrow O_1$ transitions, $0.0198 \times 2450 = 48.51$ per second. The same applies to the other two reaction steps. There is no net circulation.

The rule given in statement 24 shows that the mean lifetime, m , of a sojourn in each of the states for reaction 71 is

$$m_1 = 5 \text{ ms}$$

$$m_2 = 0.4 \text{ ms}$$

$$m_3 = 10 \text{ ms}$$

(73)

In order to provide a contrast to the reversible scheme in reaction 71, consider the case in which all transitions are irreversible, and reaction can proceed only clockwise around the cycle. Suppose this mechanism is maintained in a steady state by coupling to an energy supply, and we choose rate constants for the transitions such that the steady-state occupancies are the same as for reaction 71; these are given in equations 72. In this example, the occupancies must be proportional to the mean lifetime of each state, so a suitable choice of rate constants would be



There are two interesting respects in which the reversible (71) and irreversible (74) reactions can be compared.

7.2. Distribution of the Lifetime of an Opening

Suppose that the conductance of the two open states is identical, so they cannot be distinguished. The distribution of the duration of an opening for the reversible mechanism (71) can be shown (e.g., from equation 3.64 of Colquhoun and Hawkes, 1982) to have a probability density function

$$f(t) = 97.962e^{-t/\tau_1} + 1.037e^{-t/\tau_2} \quad (75)$$

where the time constants of the two exponential components are $\tau_1 = 10.204$ ms and $\tau_2 = 0.384$ ms. Notice that the coefficients of both terms are positive so the distribution is a monotonically decreasing curve. It has not got a maximum or even a point of inflection (see

Fig. 10a). It can be shown that this must always be true, whatever rate constants are inserted in equation 71. This result is interesting in connection with observations by Gration *et al.* (1982) of an open-time distribution that appeared to go through a maximum; their result seems to be incompatible with any equilibrium reversible reaction mechanism.

In contrast, the irreversible mechanism (74) must give a steady-state open-time distribution with a maximum. The opening must always start in state 1 and then proceed through state 2 before shutting can occur, so there are few very short openings. The pdf can again be found from equation 3.64 of Colquhoun and Hawkes (1982) or, in this case, by analogy with equation 91 below. The result for scheme 74 is

$$f(t) = 106.34(e^{-t/\tau_1} - e^{-t/\tau_2}) \quad (76)$$

where the time constants, in this case, are simply the mean lifetimes of open states 1 and 2, i.e., 9.804 ms and 0.4 ms, respectively. This distribution has a term with a negative sign and must go through a maximum (see Fig. 10a), whatever the particular values of rate constants. In these examples, the mean open lifetime is 10.2 ms for both reversible (71 and 75) and irreversible (74 and 76) cases.

7.3. Probabilities of Particular Sequences of Transitions when the Open States Are Distinguishable

Let us suppose now that open state 2 has a lower conductance than open state 1, so the two states can be distinguished on the experimental record. Such conductance substates have

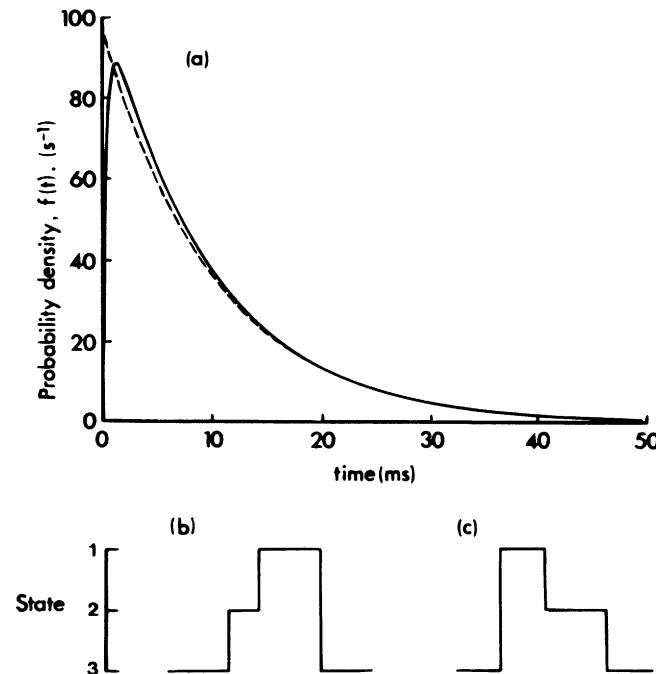


Figure 10. Reversible and irreversible mechanisms. a: The dashed line shows the pdf of the lifetime of the open (O_1 or O_2) state for the reversible mechanism in equation 71. The equation for this curve is given as equation 75. The fast component ($\tau = 0.384$ ms) of the distribution is too small in amplitude (only 1.04 s^{-1}) to be easily visible. The continuous line shows the pdf of the lifetime of the open state for the irreversible mechanism in equation 74 which is specified in equation 76. In this case, the fast component ($\tau = 0.4$ ms) has a negative sign, and the pdf goes through a maximum (i.e., very short open times will rarely be seen). b and c: Two possible types of opening for the reversible mechanism in equation 71; these will be observable only if the two open states, O_1 and O_2 , differ in conductance.

now been observed in many sorts of ion channel. Consider, for example, an opening in which state 1 is entered first, then state 2, so the sequence of transitions is $3 \rightarrow 1 \rightarrow 2 \rightarrow 3$ (see Fig. 10c). Contrast this with the sort of openings in which state 2 is entered first, then state 1, i.e., the sequence $3 \rightarrow 2 \rightarrow 1 \rightarrow 3$ (see Fig. 10b). These sequences are mirror images of each other in time, so the principle of time symmetry discussed above suggests that for any reversible mechanism at equilibrium, they should occur equally frequently.

At first sight, it is not obvious how this can happen for the mechanism in equation 71. For this mechanism, it is fairly obvious that any open period has a 98% chance of starting in state 1 and only a 2% chance of starting in state 2, because the opening rate constants are 98 s^{-1} and 2 s^{-1} (in general, such probabilities can be found from equation 3.63 of Colquhoun and Hawkes, 1982). It is nevertheless true that the above sequences are equally probable. This can easily be shown by calculation of the probabilities (π_{ij} values) that a channel in one state (i) will move next to another (j); this sort of argument has already been illustrated in Section 4, equations 33–37.

Consider first the $1 \rightarrow 2 \rightarrow 3$ transition. For the values in equation 71, the probability that a channel in state 1 will next move to state 2 is $\pi_{12} = 100/(100 + 100) = 0.5$, and the probability that once in state 2 it will move to state 3 is $\pi_{23} = 50/(50 + 2450) = 0.02$. The probability of the $1 \rightarrow 2 \rightarrow 3$ sequence is therefore $\pi_{12}\pi_{23} = 0.5 \times 0.02 = 0.01$. Now the probability that the opening starts in state 1 in the first place is 0.98, so a fraction 0.98×0.01 , i.e., 0.98%, of all openings will be of the $3 \rightarrow 1 \rightarrow 2 \rightarrow 3$ type shown in Fig. 10c. Similarly, the probability of a $2 \rightarrow 1 \rightarrow 3$ sequence is $\pi_{21}\pi_{13} = 0.98 \times 0.5 = 0.49$. However, only 2% of openings start in state 2, so a fraction 0.49×0.02 , i.e., 0.98%, of all openings should be of the $3 \rightarrow 2 \rightarrow 1 \rightarrow 3$ type shown in Fig. 10b. This is exactly the same fraction as for the mirror-image sequence, as predicted.

There have been some reports of asymmetry in sublevel structure (e.g., Hamill and Sakmann, 1981; Cull-Candy and Usowicz, 1987), though the majority of cases where the question has been inspected show no sign of asymmetry (e.g., Howe *et al.*, 1991; Gibb and Colquhoun, 1992; Stern *et al.*, 1992). The finding of asymmetry suggests that either the reaction mechanism is not reversible or that it is not at equilibrium (see, for example, Läuger, 1985; Chapter 23 this volume). Clearly, the flow of ions through an open channel is far from equilibrium, so any coupling between ion flow and channel gating could, in principle, give rise to asymmetry.

The conclusion that has just been illustrated is quite general. For a reversible mechanism at equilibrium, any sequences that are mirror images in time should be equally frequent, and the length of time spent in each of the states should have the same distribution whether the record is read from left to right or from right to left. Furthermore, this remains true even if each of the experimentally distinguishable states actually consists of any number of indistinguishable (equal-conductance) states.

None of this is, of course, true for an irreversible mechanism. For that shown in reaction 74, it is clear that every opening will consist of a $3 \rightarrow 1 \rightarrow 2 \rightarrow 3$ sequence of transitions.

8. The Problem of the Number of Channels

It is clear that, in general, there may well be more than one ion channel in the patch of membrane from which a recording is made. This means that one cannot, in general, be sure that a particular single channel current in the recording originates from the same individual ion channel that produced the previous current pulse. This, in turn, means that

the distribution of the length of the shut periods between openings cannot be interpreted without knowledge of the number of ion channels that are present. This is very unfortunate because, insofar as there will usually be more shut states than open states, the distribution of shut times is potentially more informative than the distribution of open times.

There are at least three things that can be done about this problem: (1) make an estimate of the number of channels present and make appropriate allowance if there is more than one; (2) use recordings only from patches that have one channel (evidence for this is considered below); (3) use only the brief shut periods within a burst of openings, which may be interpretable even if interburst intervals are not. It must be said, however, that quite often none of these procedures proves to be entirely satisfactory, and lack of knowledge of the number of channels continues to be a serious problem. The procedures are now discussed in a bit more detail.

8.1. Estimation of the Number of Channels

Suppose that there are N independent channels present. The probability that r of those channels are open simultaneously should be given by the binomial distribution as

$$P(r) = \frac{N!}{r!(N-r)!} p_0^r (1-p_0)^{N-r} \quad (r = 0, 1, \dots, N) \quad (77)$$

where p_0 is the probability that an individual channel is open (this will, of course, be unknown and will be lower than the *observed* probability of being open, if more than one channel is present). In principle, the value of N can be estimated from data by comparing the distribution of simultaneously open channels with the predictions of the binomial distribution. The estimation of the binomial parameter N is, however, a problem with a notorious reputation among statisticians (see Olkin *et al.*, 1981). The problem is discussed critically, in the single-channel context, by Horn (1991). He compares seven different ways of estimating N on a series of simulated data sets with a range of parameter values.

The simplest estimate of N is just the largest number of simultaneously open channels that is seen in the record. Although this sounds crude (*it is*), other methods that might be thought of as more subtle (such as maximum-likelihood estimation of N) will often produce much the same answer. The fact is that many sorts of record contain very limited information about the size of N , so no method can extract much from them. It is obvious, for example, that when a very low agonist concentration is used on a muscle endplate, long records can be obtained without any double openings at all despite the fact that the patch contains hundreds of channels. In general, it will never be possible to estimate N when the number of channels is large and the probability of each being open is small. In this case, the binomial distribution approaches a Poisson distribution, and N becomes indeterminate (only the mean, Np_0 , can be determined). (Exactly the same problem arises in the study of quantal transmitter release.) In order to have any hope of estimating N , the experiment must be done under conditions where p_0 is as high as possible (see Horn, 1991). The problem, however, remains that p_0 is the probability of being open for *one* channel, and so it cannot be inferred directly from a record derived from an unknown number of channels.

A further problem is that it is possible that the assumptions of the binomial analysis are not met. Receptor heterogeneity is a real problem (especially in the central nervous system) for this analysis (as well as for many others). There is also a possibility that receptors

may not always be independent; e.g., the opening of one receptor might influence the opening of adjacent receptors. There have been reports of such interactions, for example, Yeramian *et al.* (1986), but most are not as convincing as this one.

8.2. Evidence for the Presence of Only One Channel

Obviously, if one or more double openings are seen, there must be more than one channel. If, on the other hand, the observed record consists entirely of periods with either zero or one channel open, then there may be only one channel present. If there is a channel open for most of the time, and yet no double openings are seen, then it is fairly obvious that all the openings must come from the same ion channel. This is the basis for determining the fraction of time for which an individual channel is open by looking at clusters of channel openings at high agonist concentrations (see, for example, Sakmann *et al.*, 1980; Sine and Steinbach, 1987; Colquhoun and Ogden, 1988). If, however, much of the time is spent with no channels open, it will not be obvious how many channels are present, and some sort of statistical test is desirable. Horn (1991) suggests, on the basis of his binomial simulations, that if no double openings are seen, and the channel is open for more than about 50% of the time, then it is very likely that one channel is present. Some variants on this approach will now be discussed.

8.2.1. A Simple Approximation

Suppose that (1) channels can exist in two states only, open and shut, as in equation 1, that (2) we observe n_o single openings but no double openings, and that (3) for most of the time no channel is open; i.e., if we denote the observed mean (singly) open time m_o , and the observed mean shut time as m_s , then we assume $m_s \gg m_o$. How probable is this observation if there are actually N independent channels present? If we start with one channel open, the probability, π , that the next transition is the shutting of this one channel, with rate α , rather than a second channel opening, with rate $(N - 1)\beta'$, is

$$\pi = \frac{\alpha}{\alpha + (N - 1)\beta'} \quad (78)$$

The observed probability of being open in the experimental record, P_{ON} say, is

$$P_{ON} = \frac{m_o}{m_o + m_s} \quad (79)$$

Furthermore, given our assumption that $m_s \gg m_o$, the rate constants in this can be estimated from the data as

$$\begin{aligned} \hat{\alpha} &\approx 1/m_o \\ \hat{\beta}' &\approx 1/Nm_s \end{aligned} \quad (80)$$

so we can estimate π from the observations as

$$\begin{aligned}\pi &\approx \frac{1}{1 + \left(\frac{N-1}{N}\right) \frac{m_o}{m_s}} \\ &= \frac{1 - P_{\text{ON}}}{1 - P_{\text{ON}}/N}.\end{aligned}\quad (81)$$

We can now ask how many consecutive single openings are likely to be seen when there is actually more than one channel present. If we note that the probability that the singly open channel is followed by a transition to a doubly open channel is $(1 - \pi)$, then the probability of getting r single openings before the first multiple opening occurs (given that there is at least one single opening) is

$$P(r) = \pi^{r-1}(1 - \pi) \quad (82)$$

This is a geometric distribution of the sort already encountered in equation 36. The mean number of consecutive single openings, m , is, as in equation 38, thus

$$m = \frac{1}{1 - \pi} = \frac{1}{P_{\text{ON}}} \left(\frac{N}{N-1} \right) (1 - P_{\text{ON}}/N) \quad (83)$$

We have observed n_o consecutive single openings, so the run of single openings must be at least n_o in length. The probability of observing n_o or more single openings is, as in equation 37,

$$P(r \geq n_o) = \pi^{n_o-1} \quad (84)$$

This result can also be derived, under the above assumptions, as the approximate probability that the waiting time until the first double opening is greater than the length, T , of the observed record, given that N channels are present.

Consider, for example, a record consisting of single openings of mean length $m_o = 1$ ms and mean shut time $m_s = 99$ ms, so $m_o/m_s = 0.0101$, and $P_{\text{ON}} = 0.01$. On the hypothesis that there are actually $N = 2$ channels present, equation 81, gives $\pi = 0.9949749$. If we observe $n_o = 300$ openings (i.e., about a 30-s record) with no double openings, then equation (84) gives the probability of a run at least this long as 0.222 (or 0.134 if $N = 3$). The observation would not be surprising if there were actually two or three channels present, even though no multiple openings have been observed, so the data are insufficient to provide good evidence for the hypothesis that there is only one channel present. On the other hand, if $n_o = 1200$ single openings were observed (2 min with no double openings), equation (84) would give the probability of a run at least this long as 0.0024 if there were two channels present (or 0.0003 if three channels were present), so it is unlikely that more than one channel is functioning.

8.2.2. A Better Approximation

The case of $N = 2$ channels is discussed in detail by Colquhoun and Hawkes (1990). They give an approximation for the mean number of openings in a run of single openings as

$$m \approx \frac{2}{P_{O_2}} (1 - 0.5P_{O_2} - 0.75P_{O_2}^2) \quad (85)$$

which differs from the result in equation (83) only by virtue of the $0.75P_{O_2}^2$ term. In either case, the result approaches $2/P_{O_2}$ when the observed probability of being open, P_{O_2} , is sufficiently small. The result in equation (85) is plotted in Fig. 11, together with approximate upper confidence limits for the number of openings per run.

8.2.3. Exact Solutions

Colquhoun and Hawkes (1990) also present exact calculations concerning the lengths of runs of single openings (and bursts) in the case where there are $N = 2$ identical independent channels. Such calculations are needed to explore the conditions under which the approximations are adequate, though in order to obtain exact results it is necessary to specify the channel mechanism (whereas the above approximations have the virtue that this is not necessary). The approximation works well when (1) the openings occur singly and are well separated from each other, and (2) the openings occur in compact, well-separated bursts, the shut times within the bursts being brief relative to the openings. In the latter case, the word "opening" in the approximate argument should be replaced by "burst"; the burst is open for a large proportion of the time (so that two overlapping bursts will certainly produce a double-amplitude event), and for the purposes of the present argument (as well as for physiological purposes) the burst is the "effective opening." However, in cases where the shut times within bursts are of the same order of magnitude as the open times, the approximation may be poor.

8.2.4. Problems of Prolonged Bursts, Desensitization, and Sleepy Channels

The approximation presented above works well when openings occur singly or in compact bursts, but it would probably not be very good for channels such as the NMDA-

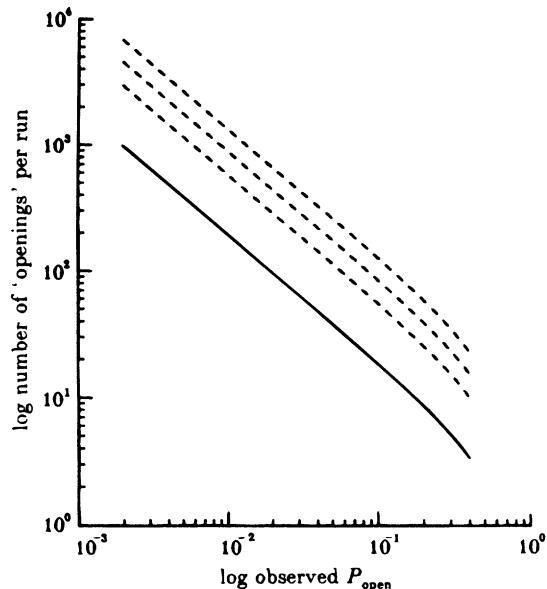


Figure 11. The mean number of openings per run of single openings in a membrane patch that contains two channels, as calculated from the approximation given in equation 85. The dashed lines show the approximate upper confidence limits for the number of openings per run, for $P = 0.05, 0.01$, and 0.001 . Reproduced with permission from Colquhoun and Hawkes (1990).

type glutamate receptor, which produce complex and prolonged burst-like channel activations containing shut periods some of which are considerably longer than the openings.

Furthermore, most channels show desensitization, inactivation, or ‘sleeping’ phenomena, which involve entry into shut states that may have very long lifetimes. If, for example, a patch contains two channels, but one of them is desensitized at the beginning of the recording, it will appear that only one channel is present. If the channel is open for much of the time, then it will be obvious when the second channel emerges from its desensitized state, because double openings will be seen straight away, but if the probability of being open is low, it may not be at all obvious that a second channel has appeared. Clearly, though, any method for trying to estimate N will not work well if N is effectively changing during the recording. This is probably one of the most serious problems in practice.

8.2.5. Fitting with a Known Number of Channels

If an estimate of the number of channels can be made, then it is possible to fit some sorts of distribution even when records contain more than one channel open at the same time (Jackson, 1985; Horn and Lange, 1983). These methods are discussed in Chapter 19 (this volume).

8.3. Use of Shut Periods within Bursts

Most channels seem to produce openings in bursts rather than singly. This observation implies only that there is more than one shut state (see Sections 4, 5, and 13; Colquhoun and Hawkes, 1982). Regardless of the mechanism, it is likely, if the gaps within a burst are short, that all of the openings in one burst originate from the same individual channel, even if there are several channels present so the next burst may originate from a different channel. In this case, the distribution of the lengths of shut periods within (but not between) bursts can be interpreted in terms of mechanism as though only one channel was present, even when it is not known how many channels are actually present. This procedure was employed, for example, by Colquhoun and Sakmann (1985) and Sine and Steinbach (1986).

9. Distribution of the Sum of Several Random Intervals

Many problems involve finding the distribution of the sum of two or more random intervals, for example, the durations of the sojourns in the various states that constitute a burst of openings. This sort of problem also arises when we consider the relationship between single-channel currents and macroscopic currents (see Section 11). Some useful examples will be discussed in this section.

9.1. The Sum of Two Different Exponentially Distributed Intervals

By way of an example, consider again the simple two-state mechanism specified in equation 1, namely:

$$\text{Shut} \xrightleftharpoons[\alpha]{\beta'} \text{Open} \quad (86)$$

What is the distribution of the time interval between two successive openings? This time interval consists of one open time plus one shut time. The pdf of the open time is $f_1(t) = \alpha e^{-\alpha t}$, and that of the shut time is $f_2(t) = \beta' e^{-\beta' t}$. We wish to know the pdf, $f(t)$, for one open time plus one shut time. Suppose that the open time is of length τ ; if the total length of the gap between openings is t , then the length of the following shut period must be $t - \tau$. Since it is a basic characteristic of our random process that events occurring in nonoverlapping time intervals are independent, we can simply multiply the corresponding probability densities, which gives $f_1(\tau)f_2(t - \tau)$. However, the length, τ , of the opening may have any value from 0 to t , so to obtain the pdf, we must sum over these possibilities. This summation, because τ is a continuous variable, must be written as an integral, so we obtain the pdf of the time between openings as

$$f(t) = \int_{\tau=0}^{\tau=t} f_1(\tau)f_2(t - \tau)d\tau. \quad (87)$$

This form of integral is called a *convolution* (of f_1 and f_2).*

This argument leads to the general rule that the pdf of a sum of random intervals is the convolution of their individual pdfs. In this case, with simple exponential pdfs, the convolution (equation 87) can easily be integrated directly. In general, however, it is much easier to solve this sort of problem by use of the Laplace transform of the pdfs, because simple multiplication of the transforms corresponds with convolution in the time domain. This is the method that must be used for a more general treatment (see Section 13; Colquhoun and Hawkes, 1982), which provides another reason to discuss a simple example now.

We shall denote the Laplace transform of $f(t)$ as $f^*(s)$. In this example, we have

$$f_1(t) = \alpha e^{-\alpha t} \quad \text{and} \quad f_2(t) = \beta' e^{-\beta' t} \quad (88)$$

so their Laplace transforms, which can be obtained from tables (e.g. Spiegel, 1965), are

$$f_1^*(s) = \alpha/(s + \alpha) \quad f_2^*(s) = \beta'/(s + \beta'). \quad (89)$$

The Laplace transform of the required pdf (equation 87) is therefore

$$f^*(s) = f_1^*(s)f_2^*(s) = \frac{\alpha\beta'}{(s + \alpha)(s + \beta')} = \frac{\alpha\beta'}{\alpha - \beta'} \left(\frac{1}{s + \beta'} - \frac{1}{s + \alpha} \right) \quad (90)$$

Inversion of this transform gives the required pdf as

$$f(t) = \frac{\alpha\beta'}{\alpha - \beta'} (e^{-\beta't} - e^{-\alpha t}) \quad (91)$$

*In general, the integral for a convolution would be from $-\infty$ to $+\infty$, but in this case the pdfs are zero for times less than zero.

Notice that this pdf is the difference between two exponential terms and therefore, unlike the simple exponential, goes through a maximum (as already illustrated; see equation 76 and Fig. 10a). This shape indicates a deficiency of very short values (compared with a simple exponential distribution), and this is what would be expected intuitively, because in order to get a short interval *both* the open and shut times must be very short, and this is relatively unlikely to happen. This characteristic shape is illustrated again in Section 11, Fig. 16, when the relationship between single-channel currents and macroscopic currents is discussed. The mean of this pdf, the mean time between openings, is, from equation 23,

$$\text{mean} = \int_0^\infty t f(t) dt = \frac{1}{\alpha} + \frac{1}{\beta'}. \quad (92)$$

As expected, this is merely the sum of the mean open time and the mean shut time. The mean opening frequency is the reciprocal of this, i.e.,

$$\frac{\alpha\beta'}{\alpha + \beta'} = \alpha p_1(\infty) = \beta' p_2(\infty) \quad (93)$$

where $p_1(\infty)$ and $p_2(\infty)$ are the equilibrium probabilities (or fractions) of open and shut channels, respectively. In other words, the mean opening frequency is the opening transition rate, β' , multiplied by the probability, $p_2(\infty)$, that a channel is shut (i.e., available to open). It is, of course, equal to the mean equilibrium shutting frequency, $\alpha p_1(\infty)$. This provides another way of interpreting rate constants in terms of the frequency with which transitions occur (see also Sections 1.2 and 4.6 and Fig. 6).

9.2. The Distribution of the Sum of n Exponentially Distributed Intervals

As an example, consider the case where we wish to know the distribution of the total open time in a burst of openings that contains exactly n openings (this will be close to the burst length if the shut periods are short). Suppose that each of the openings has the same exponentially distributed length with mean $1/\alpha$, i.e., they have pdf $f_1(t) = \alpha e^{-\alpha t}$, as above. We need, according to the argument in the previous section, the n -fold convolution of $f_1(t)$ with itself. This is made easy by using Laplace transforms as in equations 89 and 90. The Laplace transform of the required result is

$$f^*(s) = [f_1^*(s)]^n = \left(\frac{\alpha}{s + \alpha} \right)^n \quad (94)$$

Inversion of this transform gives the required pdf as

$$f(t) = \frac{\alpha(n\alpha t)^{n-1} e^{-\alpha t}}{(n-1)!}. \quad (95)$$

This is known as a *gamma distribution*. It has a mean n/α , simply n times the lifetime of an individual interval, as expected, and a variance n/α^2 . Like the result in equation 91, it is zero at $t = 0$ and goes through a maximum at $t = t_{\max} = (n - 1)/\alpha$. For $n = 1$ it reduces

to a simple exponential, but as n gets larger, the pdf becomes more and more symmetrical, eventually approaching a Gaussian shape. The cumulative form of this distribution is given, for example, by Mood and Graybill (1963):

$$F(t) = 1 - \sum_{r=0}^{n-1} \frac{(\alpha t)^r}{r!} e^{-\alpha t} \quad (96)$$

9.3. The Distribution of a Random Number of Exponentially Distributed Intervals

The results in the last section referred to the sum of a *fixed* number of exponentially distributed values. In the case of, for example, a burst of channel openings, the number of openings is not fixed but random. In the simplest cases the number of openings per burst will follow a geometric distribution, as exemplified in Sections 4.6 and 8.2. If we write the geometric distribution in the form already used in equation (82), the probability of there being r intervals (e.g., r openings per burst) is

$$P(r) = \pi^{r-1}(1 - \pi) \quad (97)$$

with mean

$$m_r = 1/(1 - \pi). \quad (98)$$

The required pdf can be found by weighting the pdf for r openings, with Laplace transform $f_1^*(s)^r$, as in equation 94, with $P(r)$ from equation 97. This gives

$$\begin{aligned} f^*(s) &= \sum_{r=1}^{r=\infty} P(r)[f_1^*(s)]^r \\ &= \frac{1 - \pi}{\pi} \sum_{r=1}^{r=\infty} \left(\frac{\pi\alpha}{s + \alpha} \right)^r \\ &= \frac{\alpha(1 - \pi)}{s + \alpha(1 - \pi)} \end{aligned} \quad (99)$$

Comparison of this result with that in equation 89 shows that its inverse transform is a simple exponential with mean $1/\alpha(1 - \pi) = m_r/\alpha$, i.e., simply the mean number of intervals, m_r , from equation 98, times the mean length of one interval; thus,

$$f(t) = (\alpha/m_r) \exp(-\alpha t/m_r) \quad (100)$$

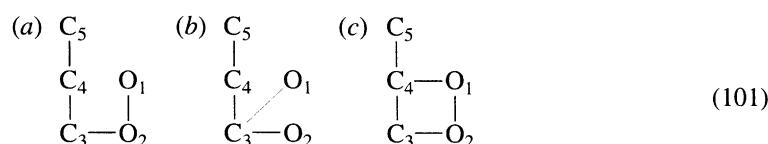
This completes the derivation of the result already given in equation 28.

10. Correlations and Connectivity

It seems surprising, at first sight, that a memoryless process can show a correlation between the length of one opening and the length of the next. Nevertheless, this is the case, as was first pointed out by Fredkin *et al.* (1985). The existence of such correlations is of importance in two main respects. First, the behaviour of channels after a perturbation (e.g., a voltage jump or concentration jump) depends on the nature of correlations (see also Section 11). And second, correlation phenomena can potentially give information about the way that the various states in the mechanism are connected. This latter ability is of considerable interest for the investigation of mechanisms, though its full potential has yet to be exploited experimentally. Both macroscopic and, to a greater extent, single-channel experiments can give information about the *number* of states that exist, but it is much harder to discover how these states are connected to each other, and the ability of correlation measurements to provide such information is a unique advantage of being able to measure the behaviour of single molecules.

10.1. Origins of Correlations

According to our (Markov) assumptions, the duration of a sojourn in any individual state must be independent of (and therefore not correlated with) the length of the sojourn in the previous state. It is for this reason that no correlations between open or shut times would be expected for the simple two-state mechanism in equation 1 or, indeed, for any of the mechanisms that have been discussed so far. In fact, correlations can arise only if there are at least two indistinguishable shut states and at least two *indistinguishable* open states (i.e., at least two open states with the same conductance). Furthermore, there must be at least two routes from open states to shut states before correlations are expected (Fredkin *et al.*, 1985; Colquhoun and Hawkes, 1987; Ball and Sansom, 1988a). More precisely, correlations will be found if there is no single state, deletion of which totally separates the open states from the shut states. The number of states that must be deleted to achieve such a separation is the *connectivity* of open and shut states, so correlations will be seen if the connectivity is greater than 1. The mechanisms in schemes 101 each have two open states (denoted O) and three shut states (denoted C).



In schemes *a* and *b* there will be no correlations; deletion of state C₃ (or of state O₂) in *a* separates the open and shut states, as does deletion of C₃ in *b*. In *c*, on the other hand, the connectivity is 2 (e.g., deletion of C₃ and C₄ will separate open and shut states), so correlations between open times may be seen. Even in this case, correlations between successive open times will be seen only if the two open states, O₁ and O₂ have different mean lifetimes. The correlations result simply from the occurrence of several C₄ ⇌ O₁ oscillations followed by a C₄ → C₃ transition and then several C₃ ⇌ O₂ oscillations, so runs of O₁ and runs of O₂

openings occur. The effect will clearly be most pronounced if the $C_4 \rightleftharpoons C_3$ reaction is relatively slow.

For example, most of the properties of the nicotinic receptor are predicted well by c : in this case O_2 has a long mean lifetime compared with O_1 (but it has the same conductance), whereas C_3 has a very short lifetime. Thus, long open times tend to occur in runs (so there is a positive correlation between the length of one opening and the next), but long openings tend to occur adjacent to short shuttings, giving a negative correlation between open time and subsequent shut time (Colquhoun and Sakmann, 1985).

These results can be extended to correlations between the lengths of bursts of openings and between the lengths of openings within a burst (Colquhoun and Hawkes, 1987). There will be correlations between bursts when the connectivity (as defined above) between open states and *long-lived* shut states is greater than 1. There will be correlations between openings within a burst when the *direct* connectivity between open states and *short-lived* shut states is greater than 1 (the term *direct connectivity* refers only to routes that connect open and short-lived shut states directly, not including routes that connect them indirectly via a long-lived shut state, entry into which would signal the end of a burst). Thus, for the examples in scheme 101, taking C_5 to be the long-lived shut state, neither a nor b would show any such correlations, whereas c would show correlations within bursts but no correlations between bursts (as observed experimentally by Colquhoun and Sakmann, 1985). The following scheme (in which C_5 and C_6 both represent long-lived shut states), on the other hand, would show all three types of correlation.



10.2. Measurement and Display of Correlations

Correlations of this sort have been reported for many other ion channels, by, for example, Jackson *et al.* (1983), Labarca *et al.* (1985), Ball *et al.* (1988), McManus *et al.* (1985), Blatz and Magleby (1989), Magleby and Weiss (1990b), and Gibb and Colquhoun (1992).

In the earlier work in this field, it was usual to measure correlation coefficients from the experimental record. However, it is visually more attractive, and in some respects more informative, to present the results as graphs, as suggested by McManus *et al.* (1985), Blatz and Magleby (1989), Magleby and Weiss (1990b), and Magleby and Song (1992). An example of such a plot is shown in Fig. 12. This graph illustrates correlations found for the NMDA-type glutamate receptor (Gibb and Colquhoun, 1992). To construct this graph, five contiguous shut-time ranges were defined (each centered around the time constant of a component of the shut-time distribution). Then, for each range, the average of the open times was calculated for all openings that were adjacent to shut times in this range, and this average open time was plotted against the mean of the shut times in the range. The graph in Fig. 12 shows a continuous decline, so it is clear that long open times tend to be adjacent to short shut times, and vice versa.

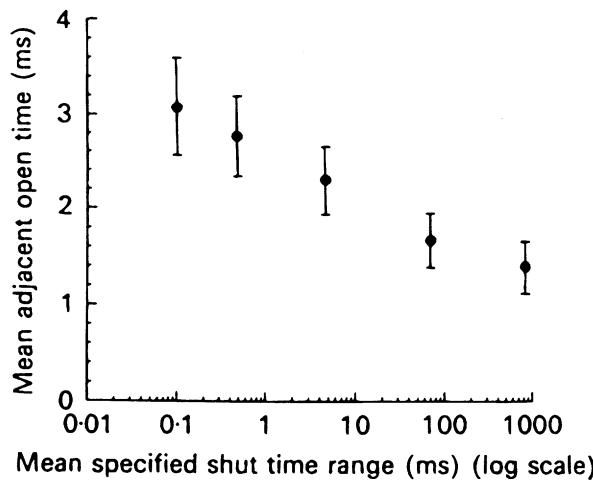


Figure 12. Relationship between the mean durations of adjacent open and shut intervals. The graph shows the mean (\pm standard deviation of mean) of the open times in 16 different patches plotted against the average of the mean adjacent shut time ranges used in each patch. Long open times tend to be next to short shut times. These results are for NMDA-type glutamate receptors in dissociated cells of adult rat hippocampus (CA1 region), activated by low glutamate concentrations. Reproduced from Gibb and Colquhoun, 1992.

10.3. Correlations as a Test of Markov Assumptions

The reason the openings that are adjacent to short closings tend to be long was investigated further, with the results shown in Fig. 13 (Gibb and Colquhoun, 1992). Figure 13A,B shows the conditional distributions of open times for openings that occur adjacent to the shortest closings (in A) and for openings that occur adjacent to the longest closings (in B). (The means from these distributions contribute points to Fig. 12.) The distributions are displayed as distributions of log(duration), as explained in Chapter 19 (Section 5.1.2) (this volume). The dashed line in A shows the (scaled) fit from B, and the dashed line in B shows the (scaled) fit from A. It can be seen that there is an excess of long openings in A, and an excess of short openings in B. This is shown quantitatively in Fig. 13C,D; it is clear from Fig. 13C that the time constants for the open-time distribution are much the same for all openings, regardless of whether they are adjacent to short or long shuttings. The mean open times differ only because the areas attached to each time constant differ, as shown in Fig. 13D. Similar observations were made by McManus and Magleby (1989) for the large-conductance calcium-activated potassium channel; they pointed out that this behaviour is a clear prediction of the Markov assumptions, whereas at least some non-Markov models do not predict such behaviour and can therefore be rejected on the basis of these observations (see Section 1.3).

10.4. Two-Dimensional Distributions

In order to extract all the information from the experimental record, it is necessary, if correlations are present, to consider two-dimensional distributions rather than the one-dimensional distributions considered so far (Fredkin *et al.*, 1985). An example of a two-dimensional distribution is shown in Fig. 14A (Magleby and Song, 1992). This distribution shows open time on one coordinate and shut time on the other. It was constructed from simulated data that were derived from the mechanism shown in equations 101c and 110, so there are two components in the open-time distributions and three components in the shut-time distributions (which resemble qualitatively the distribution shown in Chapter 19, this

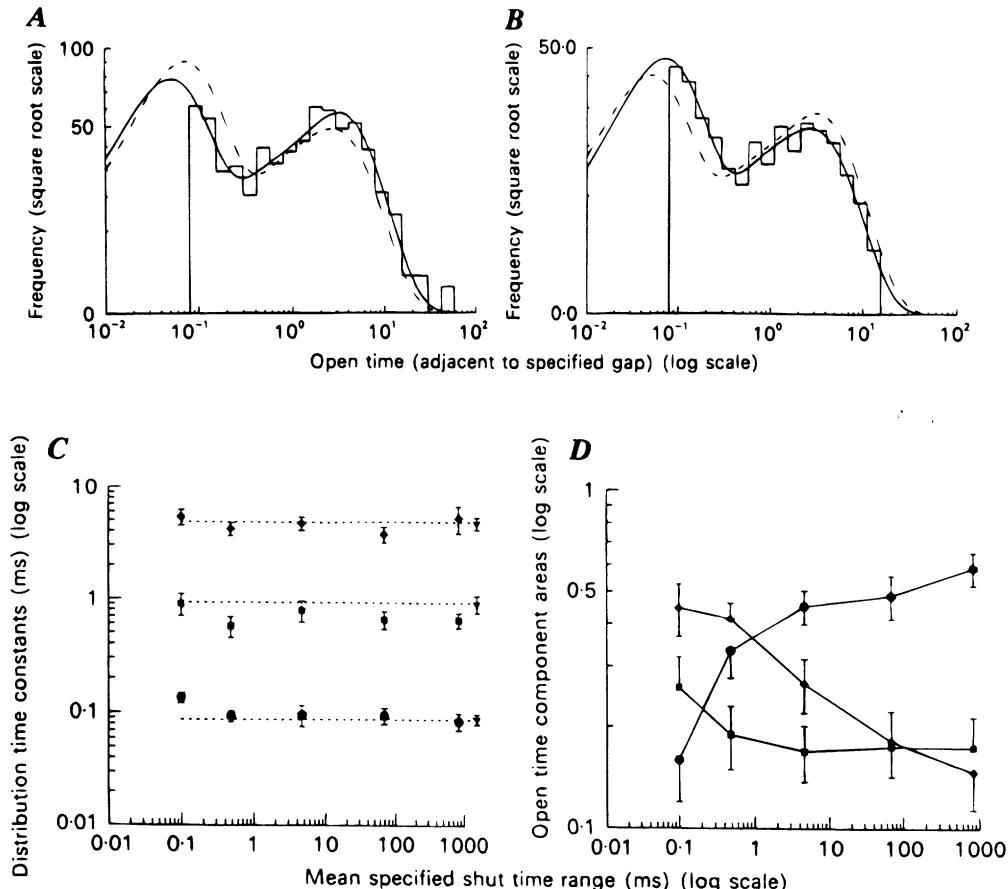


Figure 13. Conditional distributions of apparent open times adjacent to brief (A) and long (B) shut times. Data were as described in Fig. 12. A; From a total of 1206 apparent open times, 640 were identified as adjacent to shut times in the range 50 μ s to 0.3 ms. These were fitted with the sum of three exponential components (solid curve) with time constants (areas in parentheses) of 48 μ s (52%), 0.36 ms (8%), and 3.21 ms (40%). The fit predicted a total of 1154 open times. The dashed line in A shows the fit from B scaled to contain the same number of openings as the solid line. B: A total of 335 open times were identified as adjacent to shut times in the range 50–5000 ms. These were fitted with the sum of three exponentials (solid curve) with time constants (areas in parentheses) of 68 μ s (60%), 0.46 ms (5.8%), and 2.79 ms (35%). The dashed line shows the fit from A scaled to contain the same number of openings as in the solid line. The difference between dashed and solid lines indicates that, relative to openings adjacent to long shut periods, there are more long openings and fewer short openings adjacent to short shut periods. C and D: Mean time constants (C) and areas (D) of the exponential components describing conditional open-time distributions from 16 different patches. The mean (and its standard deviation) for each fitted parameter is shown plotted against the average of the mean adjacent shut-time ranges used in each patch. The inverted triangles to the right of the data values in C show the mean (and its standard deviation) for the time constants from the unconditional open-time distributions, and the dashed lines show the position of each mean across the plot. In D, the filled circles, filled squares, and filled diamonds refer to the area of the fast, intermediate, and slow components of the open-time distributions. The lines drawn in D have experimental values only at the data points and are drawn only so that the data values for each open time component can be clearly identified. Reproduced from Gibb and Colquhoun, 1992.

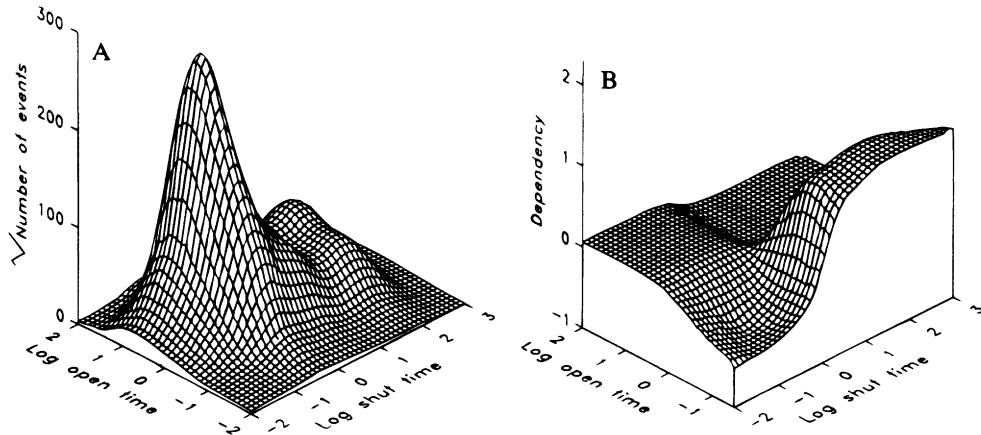


Figure 14. Illustrations of correlations based on 10^7 simulated observations (reproduced from Magleby and Song, 1990, with permission). *A:* A bivariate distribution of open time on one axis and shut time on the other. The distribution of $\log(\text{duration})$ is shown, so that peaks in the distribution occur at times corresponding to the time constants of the exponential components (see Chapter 19, this volume, Section 5.1.2). *B:* An example of the dependency plot (see text) for the same simulated data.

volume, Fig. 15). The distributions in Fig. 14 are displayed as distributions of $\log(\text{duration})$, as explained in Chapter 19 (this volume) (Section 5.1.2).

The two conditional open-time distributions that were shown in Fig. 13A,B are simply sections (at two particular fixed shut times) across the two-dimensional distribution in Fig. 14A. In practice, in order to construct the conditional distributions from experimental data, it is necessary to use a range of shut times (i.e., a shut-time bin) rather than a single exact value.

The fact that the open-time distribution differs according to the adjacent shut time (as in Fig. 13A,B) is visible in the two-dimensional distribution, but it is not very prominent. It was therefore suggested by Magleby and Song (1992) that the correlations could be made more obvious by displaying the data in the form of a *dependency plot*. They define *dependency* as the (normalized) difference between the actual frequency of particular shut-open time pairs and the frequency that would be expected if openings and shuttings were independent. Define $f_O(t_O)$ and $f_S(t_S)$ as the unconditional probability density functions for open times and shut times, respectively, and $f(t_O, t_S)$ as the two-dimensional distribution. If there were no correlations, then the two-dimensional distribution would simply be the product of the separate distributions, $f_O(t_O)f_S(t_S)$. Thus, dependency, $d(t_O, t_S)$, was defined as

$$d(t_O, t_S) = \frac{f(t_O, t_S) - f_O(t_O)f_S(t_S)}{f_O(t_O)f_S(t_S)}.$$

This will be zero for independent intervals, and a value of +0.5 would indicate that there are 50% more observed interval pairs than would be expected in the case of independent adjacent intervals. A description of how to calculate the plot from experimental values is given by Magleby and Song (1992). An example is shown in Fig. 14B for the data shown in Fig. 14A. The dependency plot clearly shows the excess of short open times adjacent to long shut times, and the deficiency of short open times adjacent to short shut-times.

Plots of the sort shown in Fig. 14 can be used to distinguish between different kinetic

mechanisms and as an aid in fitting. It may be mentioned here that full maximum-likelihood fit of the entire idealized data record, not of separate distributions, with a particular mechanism (see Sections 12.5 and 13.7) is probably the best way of extracting all the information from an experimental record. But plots like those in Figs. 13 and 14 are still good ways to display the quality of the fit so obtained, even though they are not used for the fitting process itself.

10.5. The Decay of Correlations

In a record at equilibrium, the correlation between, for example, an open time and the n th subsequent open time (for a single channel) will decay towards zero with increasing lag (n). Likewise, the distribution of open times following a jump will, after sufficient time, eventually become the same as the equilibrium distribution of all open times (see Section 11).

In principle, the connectivity between open and shut states can be measured experimentally, because the decay of the correlation coefficient with increasing lag (n) should be described by the sum of m geometric terms, where m is the connectivity minus one (Fredkin *et al.* 1985; Colquhoun and Hawkes, 1987). A similar decay should be seen in the mean lifetimes of events following a jump (Ball *et al.*, 1989). The full potential of measurements of this sort has yet to be achieved in practice.

10.6. Spurious Correlations

It was pointed out in Section 10.1 that the correlations will be strongest for the mechanism in equation 101c when the $C_4 \rightleftharpoons C_3$ reaction is relatively slow. At the extreme case, when this rate is zero, we are left with two separate channels with different mean open and shut times. Furthermore, neither of these channels would, by itself, show any correlations. Clearly, it is quite possible for spurious correlations to arise as a result of receptor heterogeneity (which is a major problem in many studies). In fact, it is even possible in principle for spurious correlations to arise even when there is more than one *identical* channel in the membrane patch (Colquhoun and Hawkes, 1987), though the importance of this has not yet been investigated. Furthermore, the inability to detect brief events may give rise to strong correlation in the observed record when there is actually little or no correlation (as exemplified in Section 12.4). In other cases imperfect resolution may attenuate real correlations (Ball and Sansom, 1988a).

11. Single Channels and Macroscopic Currents after a Jump

Essentially everything that has been said so far concerns single-channel records that are in a steady state (see Section 7). However, synapses and action potentials do not function in a steady state; they operate far from equilibrium, and so it is important to understand single-channel behaviour in the transient state before equilibrium is attained.

We shall discuss here only the case where the transition rates between states are constant, i.e., do not vary with time. This means, for example, that membrane potential and/or ligand concentration must be constant (see Section 1.1). We therefore consider only the cases where membrane potential or ligand concentration are changed in a stepwise fashion from one constant value to another. Such experiments are usually referred to as *voltage jumps* and

concentration jumps, respectively. It is, for example, common to mimic a synaptic current by applying a very brief rectangular pulse of agonist (to an outside-out membrane patch). We shall not discuss here the practical problems that often arise in achieving sufficiently rapid changes in potential or concentration to fulfill the assumptions. Some of the practical aspects are discussed in Chapter 19.

11.1. Single Channels after a Jump in the Absence of Correlations

The behaviour of single channels following a sudden change in membrane potential or ligand concentration is not necessarily the same as that in the steady state. The differences depend primarily on two things. First, they depend on the state of the system at the moment the jump was applied ($t = 0$). Second, they depend on whether the channels show correlations of the sort discussed in Section 10.

The simplest case occurs when channel openings are uncorrelated (see Section 10). This will, for example, always be the case if there is only one open state. In this case, all the openings and shuttings that follow the jump will, with one exception, have exactly the same distributions as in the steady state.

The one exception is the *first latency*. Consider, as an example, a membrane patch that is initially bathed in an agonist-free solution, so the channel(s) in it are shut. At $t = 0$ the agonist concentration is suddenly increased from zero to a finite value. The time that elapses before the first channel opening occurs is defined as the first latency, and its distribution depends on the fraction of receptors that are in each of the different shut states at $t = 0$.

Consider, for example, the simple agonist mechanism that was discussed in Section 5 and is shown again in equation 103. The channels would all be in state 3 (R, the resting state) in the absence of agonist. Compare this situation with that which obtains during a steady-state record with a constant agonist concentration: in this case, the shut channels would not all be in state 3 (R) but would be divided between state 3 and state 2 (AR), according to the value for the equilibrium constant for binding. The *initial condition* from which an opening occurs differs in these two cases, so the distribution of the shut times that precede openings will differ accordingly. This is intuitively very reasonable. At equilibrium, every shut period is preceded by an opening, so the shut period must always start in state 2 (AR), and similarly, every shut period must end in state 2 [this is why the probability $P_{22}(t)$ is needed for the derivation of the shut-time distribution given by Colquhoun and Hawkes, 1994, Appendix 1]. Because opening can occur directly from state 2, it is easy to see that the shut state preceding the next opening may be quite short; there *may* be no sojourn in state 3 before the next opening. When on the other hand, the channels are all initially in state 3 (R), the channel *must* spend time both in state 3 and in state 2 before opening is possible, and so a longer time is likely to elapse before an opening occurs.

As usual for a Markov process, these differences in the distributions depend entirely on differences in areas rather than time constants. In the case of mechanism 59, there are two shut states, so distributions of shut times are therefore a mixture of two exponentials. The time constants for the two components are the same for all shut-time distributions, including that for the first latency after a jump; but the area of the faster component will be larger for channels that were initially in state 2 (AR) than it is for channels that were initially in state 3 (R). The steady-state equivalent of this phenomenon has already been illustrated in Section 10.3 and Figs. 13 and 14.

As an example, consider the mechanism in equation 59 with the following transition rates (all in s^{-1}):



(for example, we could have $k_{+1} = 10^7 M^{-1}s^{-1}$ and a concentration of 10 μM , giving the binding rate as $100 s^{-1}$). The equilibrium shut-time distribution, calculated as described in Colquhoun and Hawkes (1994) (see also Section 13; Chapter 20, this volume) is, in the standard form of equation 25,

$$f(t) = a_1 \lambda_1 e^{-\lambda_1 t} + a_2 \lambda_2 e^{-\lambda_2 t} \tag{104}$$

where the time constants are

$$\tau_1 = 1/\lambda_1 = 0.4875 \text{ ms} \quad \text{and} \quad \tau_2 = 1/\lambda_2 = 20.51 \text{ ms} \tag{105}$$

and the areas of the components are

$$a_1 = 0.4750 \quad \text{and} \quad a_2 = 0.5250 \tag{106}$$

The mean length of a shut period at equilibrium is therefore

$$\text{mean shut time} = a_1 \tau_1 + a_2 \tau_2 = 11.00 \text{ ms} \tag{107}$$

Since, from equation 24, the mean lifetime of state 3 is 10 ms, and the mean lifetime of state 2 is 0.5 ms, it is clear that shut times consist, on average, of a $2 \rightarrow 3 \rightarrow 2$ transition (the rate constants show that it is equally likely that a channel in state 2 will, at its next transition, move to state 3 or state 1). This is for shut periods that start in state 2 and end in state 2. However, if we consider a concentration jump from zero concentration to 10 μM , the channels are initially all in state 3. The distribution of the latency until the first opening will therefore be the distribution of shut times conditional on starting in state 3. This can be found in the way given by Colquhoun and Hawkes (1994), but in this case the appropriate probability would be $P_{32}(t)$ rather than $P_{22}(t)$. The result is a distribution like that in equation 104 with the same rate constants, as given in equation 105, but with areas

$$a_1 = -0.02435 \quad \text{and} \quad a_2 = 1.02435 \tag{108}$$

and

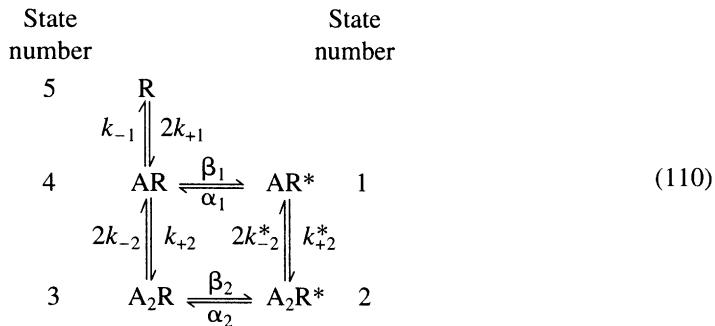
$$\text{mean shut time} = a_1 \tau_1 + a_2 \tau_2 = 21.00 \text{ ms} \tag{109}$$

In this case one of the areas is negative, which means that the distribution goes through a maximum, as illustrated earlier; in other words, very short latencies are unlikely. Correspondingly, the macroscopic current for such a jump would have a sigmoid start; the time constants for the relaxation would be, from equation 62–64, 0.3778 ms and 2.205 ms, the former having a negative amplitude.

After the first latency has elapsed, all openings and shuttings have exactly the same distributions as at equilibrium (mean open time 1.00 ms; mean shut time 11 ms). This is a consequence of the absence of correlations in this mechanism.

11.2. Single Channels after a Jump in the Presence of Correlations

The characteristics of single-channel openings after a jump when correlations are present have been considered by Colquhoun and Hawkes (1987) and by Ball *et al.* (1989). As an example, consider the mechanism in equation (101c). This has been used by several authors (e.g., Colquhoun and Sakmann, 1985) to describe the nicotinic acetylcholine receptor (see also Section 13; Chapter 20, this volume; Colquhoun and Hawkes, 1982). In this context, the mechanism may be written to show the binding of two agonist molecules (A) to the shut (R) and open (R^*) receptor, thus:



The experimental evidence suggests that the mean lifetime of open state 1 (the singly liganded open state) is considerably shorter than that of open state 2 and that the mean lifetimes of shut states 3 and 4 are short. This would account for the observed correlations, as explained in Section 10.1. An example of the calculation of single-channel properties after a jump is given by Colquhoun and Hawkes (1987) for this mechanism. They used the rate constants that were found by Colquhoun and Sakmann (1985) to provide a fair description of nicotinic receptor behaviour and used these values to predict the behaviour of channels following a concentration jump from zero to 4 nM. The time constants were, of course, the same for all distributions, but the areas changed such that the mean shut times were as follows:

- Mean latency to first opening 1539 s
- Mean shut time between first and second openings 1038 s
- Mean shut time between second and third openings 806.1 s
- Mean shut time between third and fourth openings 698.6 s

and so on, until the equilibrium mean shut time of 605.7 s is reached.

Similarly, mean lengths of the first, second, etc. openings following the jump were 0.754 ms, 1.029 ms, 1.156 ms, 1.215 ms and so on until the equilibrium mean open time of 1.267 ms was attained. The calculation of these values, for a mechanism as complex as that in equation 110, cannot be written explicitly but requires the use of matrix methods (see Section 13 below; Chapter 20, this volume; Colquhoun and Hawkes 1982, 1987). It has been shown by Ball *et al.* (1989) that such measurements can be used to provide information about mechanisms.

11.3. The Relationship between Single-Channel Currents and Macroscopic Currents

From the experimental point of view, the relationship is simple: the macroscopic current is just the sum or average of a set of single-channel records. Two schematic examples have

already been shown, in Figs. 2 and 5, of the relationship between single-channel currents and macroscopic currents. In both of these it was supposed for simplicity that the channels open synchronously at $t = 0$ or, in other words, that the first latency was negligible. This is not always true. An example of experimental measurements in which it is certainly not true is shown in Fig. 15. This shows responses of a membrane patch that contained NMDA-type glutamate receptors to application of the agonist (glutamate) for 1 ms. The patch probably contained only one channel (see Section 8), and six individual responses are shown. The average of these responses is shown at the top of the figure and is seen to follow a time course that is typical of NMDA receptors, with a relatively slow rise time followed by a slow double-exponential decay, with time constants, in this case, of $\tau = 61.5$ ms and 208 ms. In this experiment it is clear that the latency until the first opening occurs is sometimes very long indeed, and this will have a profound effect on the time course of the macroscopic current. For example, in the third trace from the top in Fig. 15, the first opening occurs about 860 ms after the 1-ms pulse, and in the fifth trace the latency is about 1340 ms.

In order to predict, from some specified mechanism, the results of an experiment like that shown in Fig. 15, we first note that the experiment involves *two* concentration jumps. First, there is a jump from zero concentration to 1 mM, the channels being initially in their resting state. This is followed, 1 ms later, by a jump from 1 mM to zero. The initial condition (i.e., the fraction of channels in each state) for the second jump is found during the calculation of the response to the first jump; it is simply the fraction of channels in each state, $p_i(t)$, at $t = 1$ ms. The methods for calculating the macroscopic (average) current have been mentioned above and are described in Chapter 20 (this volume).

11.3.1. The Simplest Example of the Effect of First Latency

In order to investigate the effect of nonsynchronous channel opening, it will be useful to consider first the simplest possible case. This case concerns a hypothetical channel that, after brief agonist application, produces an activation consisting of a single opening, after the first latency has elapsed (for the NMDA receptor, the activation is actually a great deal more complicated than a single opening). In Fig. 16A, nine examples are shown of simulated channels with a mean first latency of 1 ms and a mean open time of 10 ms (the variability of both being described by simple exponential distributions). The average current (shown at the top) is seen, not surprisingly, to have a rising phase that can be fitted with an exponential with a time constant of about 1 ms, and the decay phase has a time constant of about 10 ms. Apart from being about ten times too slow, this example is similar to what happens at a neuromuscular junction.

More surprising, perhaps, are the results shown in Fig. 16B, in which the numbers are reversed, and simulated channels have a mean first latency of 10 ms and a mean open time of 1 ms. The averaged current shown at the top is seen to have essentially the same shape as in Fig. 16A (though it is ten times smaller and considerably noisier relative to its amplitude). Thus, in this latter case, the rate of decay reflects the duration of the first latency, whereas the rate of rise represents the mean channel-open time. The reason for this result, which seems paradoxical at first sight, can be seen from the simulations (e.g., the exponential distribution of first latencies means that short latencies are more common than long ones) and from the relevant theory, which was outlined in Section 9.1. The distribution of the time from the stimulus until the channel shuts finally is simply the distribution of the sum of (1) the first latency (mean length $1/\beta'$, say), and (2) the length of the channel opening (mean length $1/\alpha$, say). This distribution has already been found, as the convolution in equation

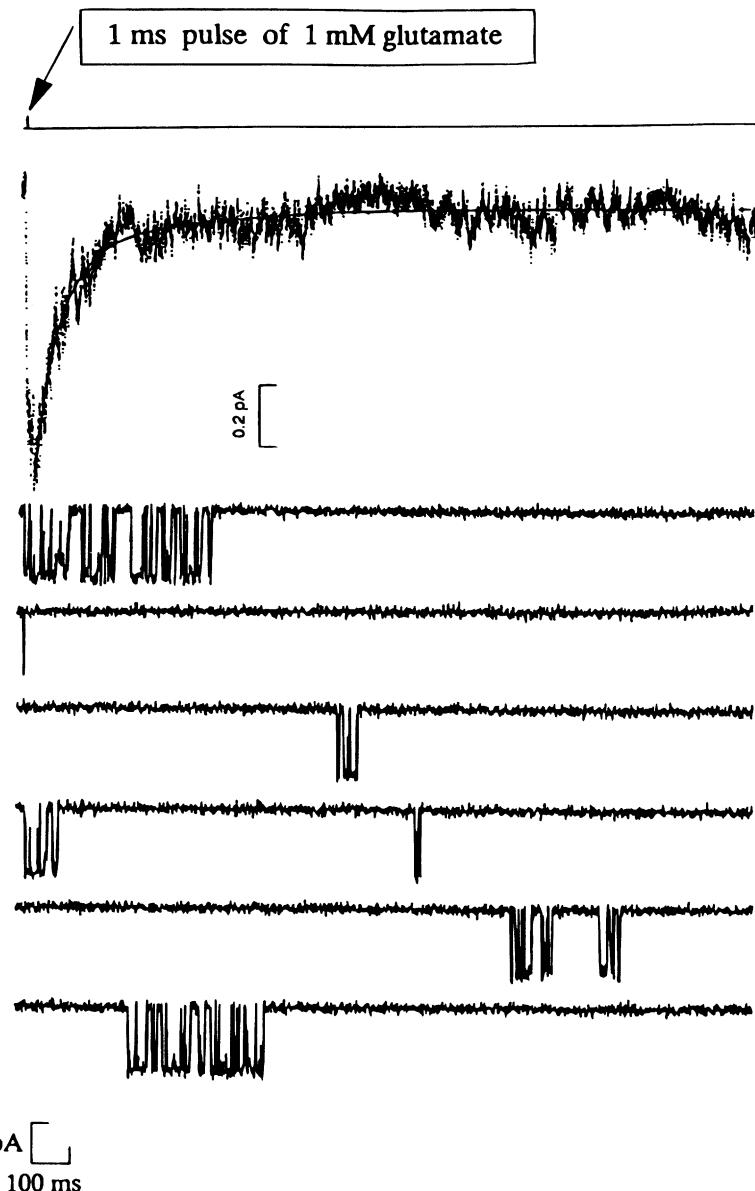


Figure 15. Illustration of first latency measurement. The lower section shows six individual responses, each 2000 ms in duration, to a 1-ms pulse of 1 mM glutamate. The time at which the command signal for the pulse was applied is shown in the topmost trace (the actual concentration change at the patch started about 1 ms later). This membrane patch contained, almost certainly, only one active channel, and it is clear that the latency before the first opening is often long (see text). The average of 122 such records is shown at the top. The decay phase of the average (starting from $t = 37$ ms) was fitted with two exponentials. Their time constants were 61.5 ms and 208 ms (the latter accounts for 23.6% of the amplitude at the starting point for the fit). (Data of B. Edmonds; outside-out patch from rat dentate gyrus granule cell at -60 mV, in solution containing 5 μ M glycine and 5 μ M CNQX. Methods as in Edmonds and Colquhoun, 1992.)

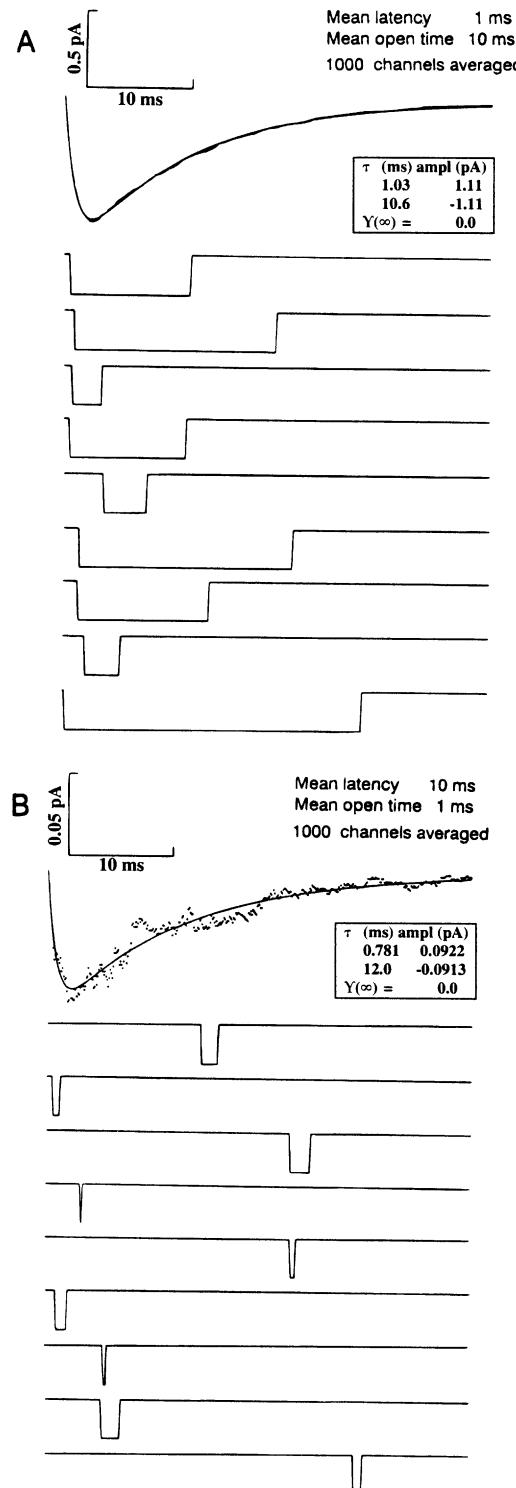


Figure 16. A simple simulation of a synaptic current in which each channel is supposed to produce only a single opening after an exponentially distributed latency. A: Mean latency 1 ms, mean open time 10 ms. The lower part shows nine examples of simulated channels. The top trace is the average of 1000 such channels; the double-exponential curve fitted to the average has $\tau = 1.03$ ms (amplitude 1.11 pA), and $\tau = 10.6$ ms (amplitude -1.11 pA). B: Similar, but with a mean latency of 10 ms and a mean open time of 1 ms. The double-exponential curve fitted to the average has $\tau = 0.76$ ms (amplitude 0.092 pA) and $\tau = 12.0$ ms (amplitude -0.091 pA).

87. Since both intervals have been taken to be simple exponentials, $f_1(t) = \alpha e^{-\alpha t}$ and $f_2(t) = \beta' e^{-\beta' t}$, the result, $f(t)$, is exactly as has already been given in equation 91. It has the form of the difference between two exponentials, and it is the curve that has been fitted to the averages in Fig. 16.

In this particular simple case, though not in general, there is a very simple relationship between the distribution, $f(t)$, of the total event length, and the shape of the averaged current. The time course of the current is given, apart from a scale factor, by the probability that a channel is open at time t . This we shall denote $P_{\text{open}}(t)$, and it can be found as follows. A channel will be open at time t if (1) the first latency is of length u , and (2) the channel stays open for a time *equal to or greater than* $t - u$. The probability that a channel stays open for a time $t - u$ or longer is, from equation 21 the cumulative distribution

$$R_1(t - u) \equiv e^{-\alpha(t-u)} \quad (111)$$

so, by an argument exactly like that used to arrive at equation 87, the probability that a channel is open at time t is

$$P_{\text{open}}(t) = \int_{u=0}^{u=t} f_2(u) R_1(t-u) du \quad (112)$$

This differs from equation 91 only by a factor of $1/\alpha$, the mean open lifetime, so

$$P_{\text{open}}(t) = f(t)/\alpha = \frac{\beta'}{\alpha - \beta'} (e^{-\beta' t} - e^{-\alpha t}) \quad (113)$$

which is, apart from its amplitude, unchanged when α and β' are interchanged. The amplitudes of the two exponential components are equal and opposite, being, from equation 113,

$$\alpha = \frac{\beta'}{(\alpha - \beta')} \quad (114)$$

with a maximum at t_{\max} , which is given by

$$t_{\max} = \frac{\ln(\beta'/\alpha)}{\beta' - \alpha}. \quad (115)$$

The simulated average currents in Fig. 16 are indeed well fitted by these values.

11.3.2. The Effect of First Latency in General

If there is more than one sort of shut state (which there invariably is for real channels), the possibility arises that the channel may open more than once after a pulse of agonist (or of membrane potential) is applied. This would, for example, be the case for the channel-block mechanism discussed in Section 4 (see Fig. 5). It is also clearly the case for the NMDA receptor as shown by the experiment in Fig. 15. If there is only one open state, then the result given above can be generalized as follows. The probability of the channel being open at time t (and hence the macroscopic current at time t) is given by the convolution of the first latency distribution with $P_{11}(t)$, where the latter was defined in equation 46 as

$$P_{11}(t) = \text{Prob}(\text{open at time } t | \text{open at time } 0). \quad (116)$$

This result has been used, for example, by Aldrich et al. (1983) and by Horn and Vandenberg (1984) for the interpretation of experiments on sodium channels, in which measurements of first latency turned out to be important for investigations of the channel mechanism.

It is important to note that $P_{11}(t)$ is the sort of probability that is used in the calculation of macroscopic currents or noise; it does *not* specify that the channel should be open *throughout* the time from 0 to t (as would be the case for analysis of single channels; e.g., see Section 4.8) but merely that it was open at 0 and at t , regardless of what happens in between. In fact, $P_{11}(t)$ describes the time course of the current that would be found by averaging single-channel records after aligning the starting points of the first opening in each record.

In general, the expression for $P_{11}(t)$ will be given by the sum of $k - 1$ exponentials that have the time constants found for macroscopic relaxations (as in equation 4) (they will be the eigenvalues of $-\mathbf{Q}$; see Section 13 and Chapter 20, this volume). These time constants will not, in general, be the same as those for any of the single-channel distributions. Thus, although the first latency distribution is a ‘single-channel quantity’, $P_{11}(t)$ is not, and there is, therefore, in general, no simple relationship between single-channel distributions and macroscopic currents.

In particular, *it is impossible to predict the response to a jump from measurements of steady-state single-channel recordings*. This is generally true, though if the single channel recordings were made under a range of conditions and were detailed enough to allow complete identification of the mechanism and all its rate constants (see Section 12), then it would of course be possible to predict the time course of macroscopic currents. This was illustrated by Edmonds and Colquhoun (1992), who show that simple averaging of aligned channel activations (measured in steady-state records) does not reproduce the shape of the macroscopic currents. However, this procedure would work, to a good approximation, for muscle-type nicotinic receptors, which produce compact bursts of openings with a very short first latency and are therefore close to the situation illustrated in Fig. 2.

If there is more than one open state, then the result stated above can be further generalized, using matrix methods, by what amounts to using a separate first-latency distribution for entry into each of the open states (see Section 13 and Chapter 20, this volume).

12. The Time Interval Omission Problem

The filtering effect of the recording apparatus, together with noise and sampling the signal at regularly spaced points in time, means that brief openings or shuttings of the ion channel will not be detectable. This will cause a distortion of the histograms of the distributions of open times and shut times that can be quite serious (see example below).

12.1. Definition of the Problem

We suppose in what follows that all events that are shorter than some fixed *resolution* or *dead time* (denote ξ_o for open times, ξ_s for shut times) are *not* detected, whereas all events longer than this are detected and measured accurately. The resolution is usually not well defined, but may be imposed retrospectively on the measurements by concatenating any

observed shut time below ξ_s with the open times on each side of it to produce one long “apparent opening.” The procedures necessary for imposition of a fixed dead time are discussed in Chapter 19 (this volume, Section 5.2). The effect on the distribution of open times that is caused by missing open times that are shorter than ξ_o is easily allowed for (see Section 6.8.1 of Chapter 19, this volume). But the concatenation of adjacent open times that occurs when the short shut time separating them is missed is potentially far more serious and may cause openings to appear to be far longer than they really are.

12.1.1. Dependence on the Method of Analysis of Experimental Records

Before any attempt can be made to make allowance for missed events, the problem must be formulated precisely. The problem is to decide how to define what it is that is *actually* measured when an experimental record is analyzed. The answer to this question will depend, to some extent, on the method that is used for the analysis. If a threshold-crossing method is used, it seems natural to define the dead time as the duration of an event that is just long enough for the signal to reach the threshold (in the absence of noise). There are two problems with this definition. First, the universal presence of noise will mean that some events that are longer than the dead time will be missed, and some events that are shorter than the dead time will be detected (see Chapter 19, this volume). Second, as pointed out by Magleby and Weiss (1990a), events that are both shorter than the dead time but are close together may sum to produce a signal that crosses the threshold. Both of these problems are less severe if the record is fitted by time-course fitting with subsequent imposition of a fixed dead time (see Chapter 19, this volume, Section 5.2).

Ideally, the method used for missed-event correction should take into account the actual properties of the method used for analysis. Draber and Schultze (1994) have made an attempt to do this (though for an analysis method that has not yet been much used in practice). The only realistic method for doing this is the (very slow) repeated simulation of the entire analysis, as proposed by Magleby and Weiss (1990a).

We now define a theoretical quantity, the *apparent* open time. This quantity is intended to be, as far as possible, what would actually be measured from an experimental record, the *observed* open time. In fact, this distinction will often be neglected, and both quantities referred to as *apparent*. The mean length of apparent openings will be denoted ${}^e\mu_o$ (where the superscript *e* stands for *effective*). For the purpose of the theory, an apparent opening is defined as starting with an opening longer than ξ_o (which is therefore visible); this is followed by any number of openings, which may be of any length but are separated by gaps that are all shorter than ξ_s and are therefore not detected; this process is ended when a shut time in excess of ξ_s is observed. Short openings, less than ξ_o are similarly treated to obtain ‘apparent shut times’. The extent to which this definition mimics reality will, as mentioned above, depend on the method used to analyze experimental records. A run of short random openings and shuttings will, from time to time, produce signals of quite unrecognizable shape, so it is impossible to anticipate all possibilities. At least it is impossible to do so in any analysis program that allows the operator to approve or disapprove the fitted durations, and, as explained elsewhere, there are good reasons, unconnected with the missed-events problem, why it is always desirable to inspect what the computer is doing to your data. There will inevitably (and probably quite rightly) be a subjective element in the operator’s response to oddly shaped signals. Fortunately, such oddities are rare in most data and so should not give rise to serious errors.

12.1.2. Dependence on the Channel Mechanism

It is an unfortunate fact that in order to make proper allowance for missed events, it is necessary to postulate a kinetic mechanism for the operation of the ion channel. When substantial numbers of both brief openings and brief shuttings are missed, very little can be done without a realistic knowledge of the mechanism, as is made clear by the discussion below. However, it is quite often the case, to a first approximation at least, that most openings are detected but many short gaps are missed (or, more rarely, the other way round). In this case, corrections can be made without detailed knowledge of the mechanism. When most openings are detected, the shut-time distribution will (apart from the lack of values below ξ_s) be quite accurate; i.e., it will have approximately the correct time constants (see Fig. 18, for example). We can, therefore, obtain a realistic estimate of the number (and duration) of missed shut times simply by extrapolating the fitted shut-time distribution to $t = 0$. This is essentially the procedure used by Colquhoun and Sakmann (1985), and it is given below (see equation 124). Even in this case, however, it was necessary to assume something about mechanisms in order to do the correction. The reason for this is that, in their data, the distribution of (apparent) open times or of burst lengths had two exponential components, so, although an estimate could be made of the number of brief shuttings that were missed, there was no way of knowing whether they were missed from 'long bursts' or from 'short bursts'. The data suggested that short bursts contained few short gaps, so, in order to perform the correction, it was assumed that *all* the missed gaps were missed from long bursts. This procedure was subsequently shown to behave quite well when tested by the exact procedures discussed below, but there can be no guarantee that it will always do so.

We shall first discuss the (oversimplified) case in which the system has only one shut state and one open state.

12.2. The Two-State Case

Suppose the true open times and shut times both follow simple exponential distributions with means μ_o and μ_s , respectively. Then we have

$$P(\text{shut time} > \xi_s) = e^{-\xi_s/\mu_s} \quad (117)$$

and so

$$\text{Mean number of openings per apparent opening} = 1/e^{-\xi_s/\mu_s} = e^{\xi_s/\mu_s} \quad (118)$$

It is well known (see Chapter 19, this volume, Sections 6.6 and 6.8) that for an exponential distribution

$$\text{Mean length of shuttings longer than } \xi_s = \xi_s + \mu_s \quad (119)$$

then

$$\text{Mean of shuttings less than } \xi_s = [\mu_s - (\xi_s + \mu_s)e^{-\xi_s/\mu_s}]/(1 - e^{-\xi_s/\mu_s}) \quad (120)$$

because $e^{-\xi_s/\mu_s} \times (\text{expression 119}) + (1 - e^{-\xi_s/\mu_s}) \times (\text{expression 120})$ must equal μ_s , the

overall mean shut time. The mean apparent open time, denoted ${}^e\mu_o$, is therefore $\xi_o + \mu_o e^{\xi_s/\mu_s} + (e^{\xi_s/\mu_s} - 1) \times$ (expression 120), as there is one less shutting than opening contributing to the apparent open time. Thus,

$${}^e\mu_o = \xi_o + (\mu_o + \mu_s)e^{\xi_s/\mu_s} - (\xi_s + \mu_s) \quad (121)$$

Similarly, the mean apparent shut time is

$${}^e\mu_s = \xi_s + (\mu_o + \mu_s)e^{\xi_o/\mu_o} - (\xi_o + \mu_o) \quad (122)$$

The values of ${}^e\mu_o$ and ${}^e\mu_s$ can be estimated from the data by averaging the observed open and shut times; ξ_o and ξ_s are known, so that equations 121 and 122 are a pair of nonlinear simultaneous equations that can be solved numerically for the true means μ_o and μ_s . For example, obtain an expression for μ_s from equation 122 and substitute it into equation 121 to obtain an equation in μ_o only, that can be solved by bisection. It turns out that these equations usually have two pairs of solutions. Suppose, for example, that $\xi_o = \xi_s = 200 \mu s$, ${}^e\mu_o = 0.6 \text{ ms}$, and ${}^e\mu_s = 2.0 \text{ ms}$. Then, there is a ‘slow’ solution ($\mu_o = 299.0 \mu s$, $\mu_s = 878.7 \mu s$) and a ‘fast’ solution ($\mu_o = 106.3 \mu s$, $\mu_s = 214.8 \mu s$). The slow solution implies, for example, that on average an observed shut time comprises 1.95 shut times separated by 0.95 (short) open times, whereas the equivalent figures for the fast case are 6.56 and 5.56. In principle, the ambiguity is not quite complete because the forms of the distributions of observed times are predicted to be different (though they have the same means) for these two solutions, but in practice the difference may be too small to be useful (Hawkes *et al.*, 1990). Furthermore with the fast solution consisting of rapid alternation of openings and shuttings of duration comparable to the resolution, the apparent openings would have the appearance of a noisy opening of reduced amplitude.

This problem has been further studied using an approximate likelihood method by Yeo *et al.* (1988), Milne *et al.* (1989), and Ball *et al.* (1990), yielding a likelihood with two almost equally high peaks. They showed that the two solutions could be resolved by making additional analyses in which ξ_o and ξ_s are changed, the real solution remains the same, and the false one is altered.

The above model, assuming fixed resolution, is used throughout this section, but Draber and Schultze (1994), following Magleby and Weiss (1990a), used a (theoretically) specified model of a detector (see above), and in the two-state problem they obtain the alternative result, in the case $\xi_o = \xi_s = \xi$,

$$\begin{aligned} {}^e\mu_o &= \frac{1}{(\mu_o - \mu_s)} \\ &\times \left\{ -\frac{2\mu_o^2\mu_s}{(\mu_o - \mu_s)} [1 - e^{(\xi/\mu_s - \xi/\mu_o)}] + \xi(\mu_o + \mu_s) - \mu_o^2 e^{(\xi/\mu_s - \xi/\mu_o)} - \mu_o \mu_s \right\} \quad (123) \end{aligned}$$

with a similar result for ${}^e\mu_s$, the subscripts o and s being interchanged. These results are close to those given by equations 121 and 122 if μ_o and μ_s are greater than about 2ξ .

12.2.1. The Case when Only Gaps Are Missed

If openings are long enough that very few are missed, then the results simplify. Thus, if $\xi_o \ll \mu_o$, equation 122 reduces to ${}^e\mu_s \approx \xi_s + \mu_s$. The openings, however, are still extended

by missing gaps, but, as μ_s is now known from this result, equation 121 can be solved for μ_o as

$$\mu_o = e^{-\xi_s/\mu_s}[(e\mu_o - \xi_o) + (\xi_s + \mu_s)] - \mu_s \quad (124)$$

Analogous results, interchanging o and s , can be obtained if shut times are long.

12.2.2. Bursts of Openings

We consider here only the case in which most openings are detected but many shut times within a burst are undetected. As before, this implies that gaps will rarely be extended by undetected openings, so if μ_g now denotes the true mean length of gaps within bursts, the observed mean length of such gaps will again be $\xi_s + \mu_g$. Since we have assumed that most openings are detectable, the mean length of the observed burst will be close to the true mean burst length. Thus, both intraburst gap lengths and burst lengths can be estimated from the data. However, the apparent openings will be longer than the true openings, and the observed number of openings per burst will be correspondingly too small. It is for this reason that Colquhoun and Sakmann (1985) presented primarily distributions of gap lengths and burst lengths but not those of apparent open times or of the number of apparent openings per burst.

Corrected means for the last two distributions can be obtained as follows for the case in which the true openings and the true gaps within a burst each have simple exponential distributions. The burst distribution is fitted to give an estimate of the mean burst length, μ_{bst} , and the number of bursts, N_{bst} , each of which should be close to the true values. The distribution of lengths of gaps within bursts is fitted to give estimates of their true length, μ_g , and of their true number, N_g , which may be considerably greater than the observed number, $n_s = N_g e^{-\xi_s/\mu_g}$. The true number of gaps per burst, μ_r , is estimated as the total number of gaps divided by the total number of bursts, so $\mu_r = N_g/N_{bst}$. The true mean open time can be estimated by noting that the mean total shut time per burst including undetected gaps, is $\mu_g\mu_r$, so

$$\mu_o = \frac{\text{mean open time per burst}}{\text{mean number of openings per burst}} = (\mu_{bst} - \mu_g\mu_r)/(\mu_r + 1) \quad (125)$$

This is essentially the correction employed by Colquhoun and Sakmann (1985).

12.3. The General Markov Model

The previous section discussed only the two-state case and was concerned only with the *means* of the apparent observed open times and of observed shut times, according to particular assumptions about how these arise from the inability to observe small intervals. We need to extend this to models of channel action with any number of shut states and open states; we also need to predict the distributions of observed quantities, not only their means. So far, this has been achieved only in the case where all open states have the same conductance. Several approximate methods have been described, for example by Blatz and Magleby (1986), Yeo *et al.* (1988), and Crouzy and Sigworth (1990), in each case approximating the distributions by mixtures of exponential distributions. An exact solution in terms of Laplace

transforms was obtained by Ball and Sansom (1988b) following earlier work by Roux and Sauvé (1985).

Hawkes *et al.* (1990) obtained the exact algebraic forms of these probability density functions in the case $\xi_o = \xi_s$; they presented some numerical examples that suggested that the best of the mixed-exponential approximations was that of Crouzy and Sigworth (1990). Unlike the distributions of true open times or shut times, they are not mixtures of exponentials but are sums of exponentials multiplied by polynomials in t ; a different form holds over different ranges of length ξ , so that over the interval $[\xi r < t < \xi(r + 1)]$ the multiplying polynomials are of degree $(r - 1)$; the density is, of course, zero for $t < \xi$. These distributions are reasonably easy to compute for small t but get progressively more complicated as t increases and eventually become numerically unstable. An alternative approach by Ball and Yeo (1994) is based on numerical solution of a system of integral equations. Ball *et al.* (1991, 1993b) obtained a solution, in terms of Laplace transforms, in the more general setting of semi-Markov processes (which includes fractal and diffusion models as well as the Markov model discussed here). Ball *et al.* (1993a) showed that a general result of Hawkes *et al.* (1990), from which the above result specific to Markov models was obtained, can be extended into this more general setting.

Jalali and Hawkes (1992a,b) (see also Hawkes *et al.*, 1992) obtained asymptotic forms for these probability densities that are extremely accurate except possibly for quite small values of t . They recommend using the exact form for $t < 3\xi$ and the asymptotic form for $t > 3\xi$. Brief details are given in Section 13.7. The asymptotic distribution not only has the form of a mixture of exponentials, but it also has the same number of exponential components as the true distribution (that which would be found if no intervals were missed). However, the values of the time constants and of their associated areas may be quite different. It is this asymptotic form that would be estimated when fitting a mixture of exponentials to experimentally observed time intervals using the methods described in Chapter 19 (this volume, Section 6.8).

We consider the mechanism of scheme 110 (see also equation 127), which has two open states and three shut states, with parameter values $\alpha_1 = 3000 \text{ s}^{-1}$, $\alpha_2 = 500 \text{ s}^{-1}$, $\beta_1 = 15 \text{ s}^{-1}$, $\beta_2 = 15,000 \text{ s}^{-1}$, $k_{+1} = 5 \times 10^7 \text{ M}^{-1}\text{s}^{-1}$, $k_{+2} = k_{+2}^* = 5 \times 10^8 \text{ M}^{-1}\text{s}^{-1}$, $k_{-1} = k_{-2} = 2000 \text{ s}^{-1}$, $k_{-2}^* = (1/3) \text{ s}^{-1}$, and agonist concentration $x_A = 0.1 \mu\text{M}$. A set of data, in the form of a sequence of open and shut times, was simulated from this model; a resolution of 50 μs (for both open and shut times) was then imposed on the record (see Chapter 19, this volume, Section 5.2) to produce a sequence of 10,240 *apparent* open times alternating with 10,240 *apparent* shut times. We will refer to this as the *simulation model* for the remainder of this section.

The true open time distribution has two exponential components with time constants 2.00 ms and 0.328 ms with corresponding areas of 0.928 and 0.072, giving an overall mean of 1.88 ms. Figure 17 shows the theoretical distribution of the logarithm of apparent open times (see Section 5.1.2 of Chapter 19, this volume), and this compares well with a histogram arising from the simulation. The true distribution of open times is shown for comparison. Compared with the true distribution, the distribution of apparent open times has been shifted to the right, having a mean of 3.52 ms rather than 1.88 ms; this shift is not caused by missing the short open times but results from missing the short shut times.

The distribution of apparent open times does not have a mixed exponential form for small t , but for $t > 3\xi$ it is very well approximated by the asymptotic distribution, which is a mixture of exponentials. This gives us another way of comparing the true and apparent distributions. First note that the pdf of apparent open times is zero below the dead time, $t = \xi$, whereas the true open time pdf starts at $t = 0$; thus, in order to compare the relative

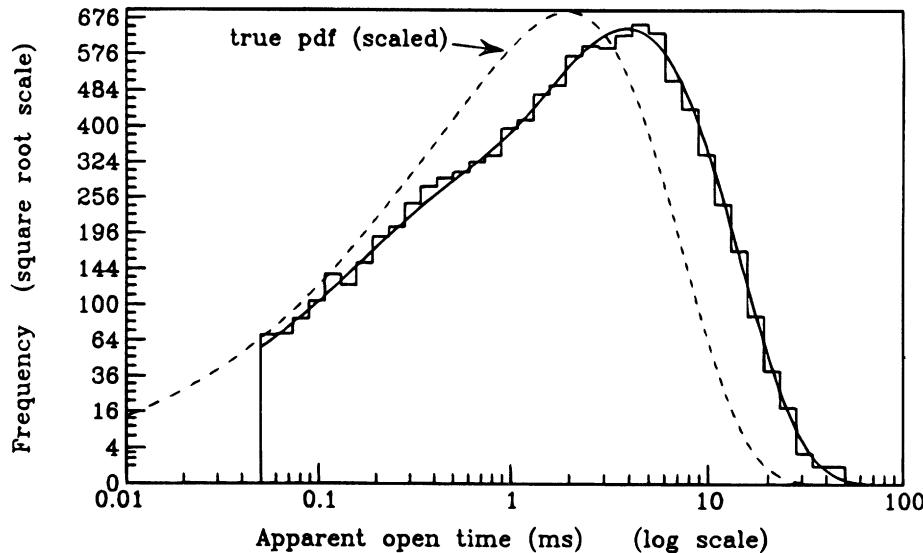


Figure 17. Probability density of the logarithm of open times for the model defined in the text. The solid line is the theoretical distribution of apparent open times for the case when the dead time is $\xi = 50 \mu\text{s}$. The histogram shows the distribution of 10,240 open times, which were simulated on the basis of this model and then subsequently had a resolution of 50 μs imposed, as described in Section 5.2 of Chapter 19 (this volume). The dashed line shows the true distribution of open times, scaled to predict the correct number of observations greater than $t = \xi$.

areas of components of the true distribution with those of the asymptotic distribution of apparent open times, it is necessary to project the exponentials of the asymptotic distribution back to $t = 0$. When this is done, we obtain an approximate distribution with time constants of 3.89 ms and 0.328 ms with corresponding areas of 0.869 and 0.131 (so the overall mean is 3.42 ms, which is close to that of the exact distribution of apparent open times). Comparing this with the true distribution, we see that the short time constant remains virtually the same but the longer one has almost doubled. This happens because, according to the mechanism used for the example, most of the short shut times occur in (and are therefore missed from) the 'long bursts' (see also Section 12.1).

Let us turn now to the distribution of shut times. The true distribution of all shut times in this example is a mixture of three exponentials with time constants of 3789 ms, 0.485 ms, and 53 μs , with areas of 0.262, 0.008, and 0.730, respectively. The distribution of apparent shut times, for $t > 3\xi$, is well approximated by the asymptotic form of this distribution, which is a mixture of three exponentials with time constants of 3952 ms, 0.485 ms and 54 μs ; the areas are 0.263, 0.008, and 0.729, respectively (when the asymptotic distribution is projected back to $t = 0$, as above). Apart from a slightly increased long time constant, this is almost identical with the true distribution. This is illustrated in Fig. 18, which shows the logarithmic plots of apparent shut times and true shut times. When the latter are scaled to consider only intervals greater than ξ , they are almost identical.

The reason for these two different types of behaviour is that the true shut time distribution has an important component, area 0.730, with a time constant of 53 μs , which is almost the same length as the dead time; many of these will be missed, leading to concatenated open times. In contrast, the shortest open time constant is more than six times the dead time, so

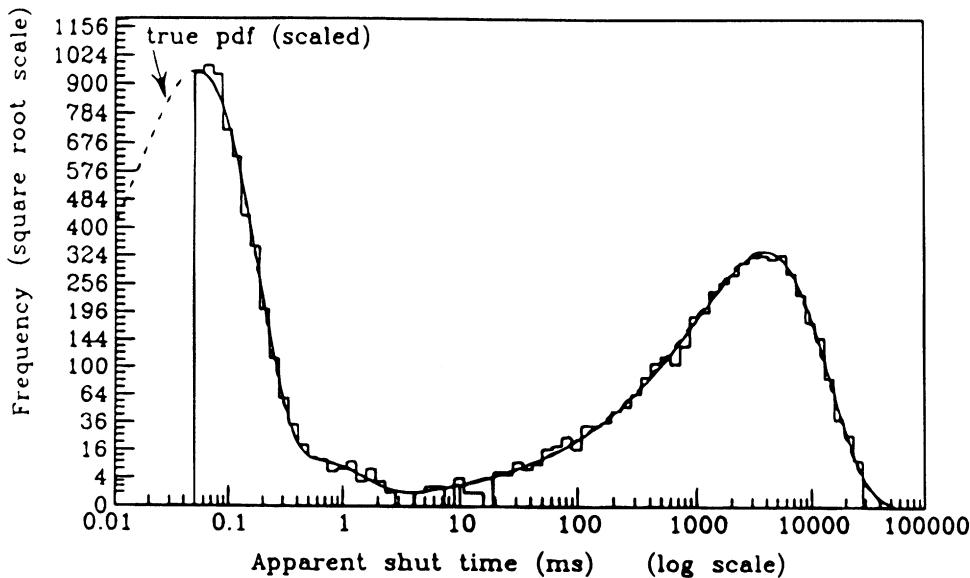


Figure 18. Probability density of the logarithm of shut times for the model defined in the text. The solid line is the theoretical distribution of apparent shut times when the deadtime $\xi = 50 \mu\text{s}$. The histogram shows the distribution of 10,240 shut times, which were simulated on the basis of this model after imposition of a 50- μs resolution. The dashed line showing the true distribution of shut times, scaled as described for Fig. 17, is virtually indistinguishable from the distribution of apparent shut times when $t > \xi$.

few open times will be missed. Thus, the distribution of shut times is almost undistorted in the sense that fitting the observed values with exponentials will give something close to the true time constants and areas (though the overall average of the observed values would be considerably increased, from 993 ms to 1855 ms, because of missing short shut times).

12.4. Joint Distributions of Adjacent Intervals

Magleby and co-workers (Blatz and Magleby, 1989; Weiss and Magleby, 1989; McManus and Magleby, 1989; Magleby and Weiss, 1990a,b) have used extensive simulation to show that the joint distribution of adjacent apparent open times and shut times can be very useful in distinguishing between different mechanisms that have very similar overall distributions of these variables when considered separately. They also use them for parameter estimation. The extra information concerning the relationship between the durations of neighbouring intervals is very valuable (see Sections 10 and 11).

The methods of Hawkes *et al.* (1992) described above can also be used to obtain the theoretical joint distributions of the adjacent apparent open and closed intervals, allowing for time interval omission. The appropriate formulas are outlined in Section 13.7; more detailed formulas and software to calculate and display these distributions are given by Srodzinski (1994).

Figure 19A shows the distribution of apparent open times that are adjacent to short shut times (i.e., those less than 150 μs) for our simulation example. Note again the good correspondence between theory and simulation. Compared with the overall distribution of apparent open times there are relatively few short open times. Figure 19B shows equivalent results for apparent open intervals adjacent to long apparent shut times (greater than 10 ms). This time we see an excess of short open times. The complementary features of these two graphs are a representation of a negative correlation between adjacent apparent open times and apparent shut times. This is illustrated another way in Fig. 19C, which shows how the mean apparent open time, calculated from the above theory, decreases for intervals adjacent to larger apparent shut times. This graph shows a continuous but very nonlinear decline. In practice, shut-time ranges must be used, as in the experimental example in Fig. 12, so the graph is not continuous. The model and parameter values used for Figs. 17 to 19 are based on observations for the frog muscle nicotinic receptor; the form of the decline for the NMDA receptor, illustrated in Fig. 12, is more linear than that plotted in Fig. 19C.

It is worth noting that time interval omission can induce a correlation not present in the true record. For example, model I of Blatz and Magleby (1989) has two open states whose mean lifetimes are almost the same; the discussion in Section 10.1 implies that there should be little correlation between adjacent *true* intervals (none at all if the means are identical). Nevertheless, there is quite strong negative correlation between *observed* open times and adjacent *observed* shut times. The reason for this can be explained with respect to a modified version of our simulation model, which then becomes a simpler version of Blatz and Magleby's model. Modify our model so that direct interchange between the two open states is impossible, and make $\alpha_1 = \alpha_2$, so the mean lifetimes of the two open states are the same. Now open state 2 is next to shut state 3, which has a mean life of 53 μs (just larger than the dead time of 50 μs) with a high probability of returning to state 2, so that successive open sojourns in state 2 are likely to be concatenated with short sojourns in state 3 to form long apparent open times, which are likely to be adjacent to short apparent shut times. State 1, however, is next to state 4, which not only has a mean life of 455 μs but is highly likely to result in a subsequent visit to the very long-lived shut state 5. Thus, open sojourns in state 1 are likely to be isolated and therefore constitute relatively short apparent open times adjacent to quite long apparent shut times. A negative correlation therefore appears between the adjacent *apparent* times, although there is none between *true* adjacent times.

12.5. Maximum-Likelihood Fitting

The ability to calculate the theoretical distributions of things that are actually observed (rather than of what would be observed if the resolution was perfect) opens the way to fitting a specified mechanism directly to the data. Previously, one could only fit empirical mixtures of exponentials separately to open times, shut times, bursts lengths, etc., but to interpret these results in terms of a mechanism and to estimate from them the values of the underlying mass-action rate constants are feasible only approximately and in simple cases. In any case, such methods are very *ad hoc* and almost certainly inefficient. It is, for example, far from obvious how to combine the (often overlapping) information from fitting various different sorts of distribution. For example, the distributions of burst length and of total open time per burst contain different but overlapping information about the burst structure in the data.

However, by using the above distributions for *apparent* open and shut times, i.e., the distributions of what is actually *observed*, it is possible to calculate the *likelihood* for an entire single-channel record, represented as an alternating sequence of open and shut times.

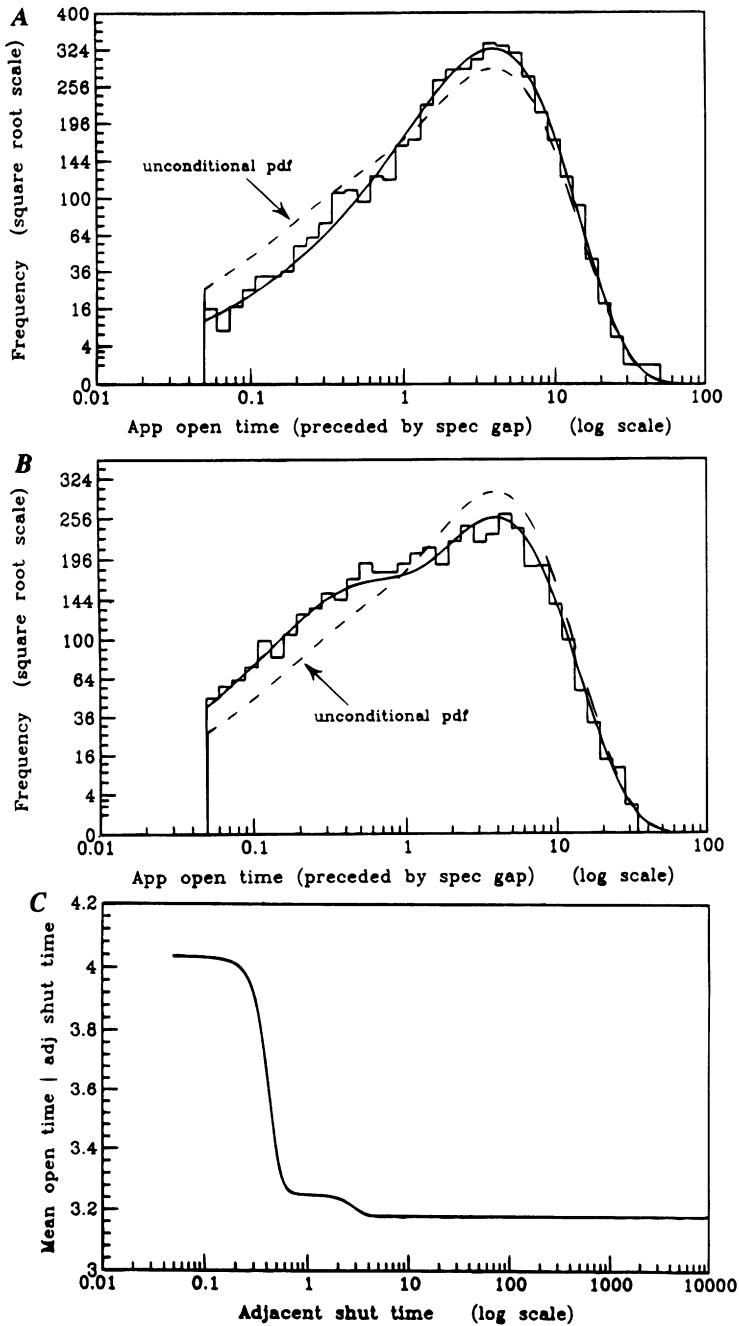


Figure 19. A shows the theoretical distribution (solid line) of apparent open times that are adjacent to short apparent shut times (less than 150 μ s). The histogram shows the distribution of simulated (see text) values of the same quantity. The dashed line corresponds to the overall theoretical distribution of apparent open times, given in Fig. 17. B shows similar results for openings adjacent to long apparent shut times (greater than 10 ms). C shows the plot of the theoretical mean of apparent open times that are adjacent to apparent shut times of a given duration, against the logarithm of the shut time.

This calculation takes the form of an enormous number of matrix multiplications, the calculation for the first opening provides the appropriate initial condition for the calculation for the subsequent shut time, which in turn provides an appropriate initial condition for the next opening, and so on up to the end of the data (see Section 13.7). Thus, the *order* in which openings and shuttings occur, and so information about correlations between the durations of neighbouring intervals, is taken into account correctly; in contrast, the separate distributions of apparent open and shut times, described above, lose this information. The calculations are done in a manner similar to that used by Ball and Sansom (1989), following earlier work by Horn and Lange (1983), assuming ideal data ($\xi = 0$).

With this approach, the parameters to be fitted are the actual mass-action rate constants in the reaction mechanism (*not* empirical time constants and areas). The values of these parameters are adjusted by a suitable search routine so as to maximize the likelihood for the entire record. We have found this to be quite feasible on a fast PC for a record consisting of several thousand intervals. Furthermore, it is possible to fit simultaneously data from several different sorts of measurement, for example, recordings made with different concentrations of agonist.

Thus, we do not have to fit separately all of the sorts of distribution mentioned above. However, it will be useful for model validation to compare observed histograms with theoretical distributions calculated from the model, especially the joint distributions of adjacent open and shut times, using values of the parameters fitted by the maximum-likelihood method.

The likelihood itself can be used to judge the relative merits of alternative postulated mechanisms. If each of the proposed mechanisms is fitted to the same data, the relative plausibility of each mechanism can be assessed from how large its maximised likelihood is; this was done, for example, by Horn and Vandenberg (1984) (without missed-event correction).

On the basis of the data from our simulation example above, the free parameters were adjusted (by a simplex method) to maximise the likelihood of the sequence. A comparison of the true and estimated parameter values is given in Table I. These agree very well, but in some cases, especially with less data, one would expect that some parameters in a mechanism would be estimated quite well and others poorly. This feature depends on the nature of the mechanism (e.g., rates leading from a state that is rarely visited will be poorly estimated) and is found in other methods of estimation (see Fredkin and Rice, 1991).

Table I. Comparison of True and Estimated Parameters from a Simulation of Mechanism 110

Parameter	True value	Estimated value ^a	Units
α_1	3000	2848	s^{-1}
α_2	500	521.4	s^{-1}
β_1	15	15.74	s^{-1}
β_2	15,000	15,592	s^{-1}
$2k_{+1}$	1×10^8	9.529×10^7	$M^{-1}s^{-1}$
k_{+2}	5×10^8	5.103×10^8	$M^{-1}s^{-1}$
k_{+2}^*	5×10^8	5.103×10^8	$M^{-1}s^{-1}$
k_{-1}	2000	1960	s^{-1}
$2k_{-2}$	4000	3919	s^{-1}
$2k_{-2}^*$	0.666667	0.7243	s^{-1}

^aNote that parameter estimates have been constrained so that $k_{-1} = k_{-2}$ and $k_{+2} = k_{+2}^*$ and microscopic reversibility is preserved.

13. A More General Approach to the Analysis of Single-Channel Behaviour

It would involve a great deal of work if the sort of analysis given for the channel-block mechanism (Section 4) had to be repeated for every type of mechanism that one wished to consider. Furthermore, it is found that the approach given above is not sufficiently general to allow analysis of some mechanisms that are of direct experimental interest. In particular, mechanisms with more than one open state and/or cyclic reactions cannot be analyzed by the relatively simple methods used so far. Consider, for example, the mechanism in equation 110, which has two open states (labelled 1 and 2) and three shut states.

We shall assume that the conductance of the two open states is the same, so, during a single opening, there may be any number of oscillations between them: $AR^* \rightleftharpoons A_2R$. Similarly a gap within a burst may involve any number of oscillations between $AR \rightleftharpoons A_2R$. The analysis is further complicated by the fact that there are two different ways in which the opening may start (via $AR \rightarrow AR^*$ or $A_2R \rightarrow A_2R^*$) and, correspondingly, two routes by which the opening may end. Clearly, the probability that an opening starts by one of these routes rather than the other will depend on how the previous opening ended. One would expect, for example, that the first opening in a burst is more likely to start via $AR \rightarrow AR^*$ than subsequent openings because the start of a burst must involve passage through AR , whereas a gap within a burst may be spent entirely in A_2R .

In Section 4.6, the distribution of the number of openings per burst was found by simple multiplication of probabilities for the routes through the burst. In the present example there are many different possible routes through a burst, and the only way in which it is practicable to find the appropriate combination of probabilities is to describe them by matrix multiplication. It turns out that matrix notation is very convenient for this sort of problem. By its use one can write down just a few equations for equilibrium single-channel behaviour (Colquhoun and Hawkes, 1982). This enables a single computer program to be written that will evaluate numerically the predicted behaviour of any mechanism, given only the transition rates between the various states. Chapter 20 (this volume) contains details of various matrix results and methods of computation; we suggest that it be read in conjunction with this section.

13.1. Specification of Transition Rates

The transition rates are most conveniently specified in a table or matrix (denoted \mathbf{Q}), with the entry in the i th row and j th column (denoted q_{ij}) representing the transition rate from state i to state j (as already defined in equation 7). This fills the whole table except for the diagonal elements ($i = j$). These, it turns out, are most conveniently filled with a number such that the sum of the entries in each row is zero. Thus, from rule 24, $-1/q_{ii}$ is the mean lifetime of a sojourn in the i th state, as is clear from the following examples. For the simple channel-block mechanism (equation 29) with $k = 3$ states, we have

$$\mathbf{Q} = \begin{bmatrix} 1 & 2 & 3 \\ -(\alpha + k_{+B}x_B) & k_{+B}x_B & \alpha \\ k_{-B} & -k_{-B} & 0 \\ \beta' & 0 & -\beta' \end{bmatrix} \quad (126)$$

Similarly, for the more complex agonist mechanism in scheme 110, with $k = 5$ states, we have

$$\mathbf{Q} = \begin{bmatrix} 1 & 2 & 3 & 4 & 5 \\ 1 & -(\alpha_1 + k_{+2}^* x_A) & k_{+2}^* x_A & 0 & \alpha_1 & 0 \\ 2 & 2k_{-2}^* & -(\alpha_2 + 2k_{-2}^*) & \alpha_2 & 0 & 0 \\ 3 & 0 & \beta_2 & -(\beta_2 + 2k_{-2}) & 2k_{-2} & 0 \\ 4 & \beta_1 & 0 & k_{+2} x_A & -(\beta_1 + k_{+2} x_A + k_{-1}) & k_{-1} \\ 5 & 0 & 0 & 0 & 2k_{+1} x_A & -2k_{+1} x_A \end{bmatrix} \quad (127)$$

where x_A is the agonist concentration. This matrix, with some specific parameter values, was used for some numerical examples in Section 12 and in Chapter 20 (this volume). Notice that these two examples illustrate the convenient numbering convention for the states that underlies the notation introduced by Colquhoun and Hawkes (1982). The open states have the lowest numbers ($1, \dots, k_{\mathcal{A}}$), and shut states have the higher numbers. For the purpose of analysis of bursts, short-lived shut states are given lower numbers than long-lived shut states. This convention allows convenient partitioning of the \mathbf{Q} matrix into subsections. This partitioning is shown explicitly in Section 2 of Chapter 20 (this volume), and is used throughout this section.

13.2. Derivation of Probabilities

The probabilities that are needed for noise and relaxation analysis, which were defined as $P_{ij}(t)$ in equation 8, can be considered as elements of a matrix, which we shall denote $\mathbf{P}(t)$. It can be found by solution of a differential equation:

$$d\mathbf{P}(t)/dt = \mathbf{P}(t)\mathbf{Q} \quad (128)$$

The solution is, quite generally,

$$\mathbf{P}(t) = e^{\mathbf{Qt}} \quad (129)$$

This has a matrix in the exponent, but its evaluation requires only operations of matrix addition and multiplication, because the exponential is defined in terms of its series expansion:

$$e^{\mathbf{Qt}} = \mathbf{I} + \mathbf{Qt} + (\mathbf{Qt})^2/2! + \dots \quad (130)$$

where \mathbf{I} is a unit matrix (unit diagonals, zeroes elsewhere). In practice, this is not the most convenient way to evaluate the exponential term (see Chapter 20, this volume); in fact, each element of $\mathbf{P}(t)$ (and hence the relaxation or the autocovariance function of noise) can be written in terms of the sum of $k - 1$ exponential terms of the form

$$P_{ij}(t) = p_j(\infty) + w_1 e^{-\lambda_1 t} + w_2 e^{-\lambda_2 t} + \dots \quad (131)$$

In this expression $p_j(\infty)$ is the equilibrium probability that the system is in state j , which $P_{ij}(t)$ must approach after a long time ($t \rightarrow \infty$). The coefficients w_i can be determined from \mathbf{Q} by the methods described in Chapter 20 (this volume; see Colquhoun and Hawkes, 1977,

1981, 1982, for details). The rate constants λ_i , which were found by solution of a quadratic in equations 30 and 53, are found, in general, as the solution of a polynomial of degree $k - 1$ that can be derived from \mathbf{Q} . They are known as the eigenvalues of \mathbf{Q} (actually, the eigenvalues of $-\mathbf{Q}$). One of the eigenvalues is zero, as \mathbf{Q} is singular, leaving only $k - 1$ further eigenvalues to find. Standard computer subroutines exist for finding them. These methods are discussed in Chapter 20 (this volume) together with the use of $e^{\mathbf{Qt}}$ in describing the relaxation of the macroscopic current toward equilibrium following a jump. General methods for the calculation of the equilibrium occupancies directly from the \mathbf{Q} matrix are given in Section 3 of Chapter 20 (this volume).

For the analysis of single channels, however, we usually need a different sort of probability, one that requires that we stay within a specific subset of states throughout the whole time from 0 to t . An example of such a probability was defined in equation 47 and explicitly derived in equations 48–54 when the distribution of the burst length for the channel block mechanism (equation 29) was considered. In that case, we specified in equation 47 that we stayed within the burst (i.e., in state 1 or 2) from 0 to t . It will be convenient to give a symbol \mathcal{E} , say, to this set of ‘burst states’ and to denote the number of such states as $k_{\mathcal{E}}$ ($k_{\mathcal{E}} = 2$ in this case). Similarly, in the case of the more complex agonist mechanism of equation 110, \mathcal{E} would consist of states 1, 2, 3, and 4, and $k_{\mathcal{E}} = 4$. Probabilities such as that in equation 47 will be denoted, by analogy with equation 8, as $P'_{ij}(t)$, in which the subscripts i and j can stand for any of the \mathcal{E} states. In the case of burst length, we can appropriately denote the $(k_{\mathcal{E}} \times k_{\mathcal{E}})$ matrix of such quantities as $\mathbf{P}_{\mathcal{E}\mathcal{E}}(t)$, and it is given quite generally by

$$\mathbf{P}_{\mathcal{E}\mathcal{E}}(t) = e^{\mathbf{Q}_{\mathcal{E}\mathcal{E}} t} \quad (132)$$

where $\mathbf{Q}_{\mathcal{E}\mathcal{E}}$ is the submatrix of \mathbf{Q} relevant to the burst states. In the case of the simple channel-block mechanism, for example, this is the top left-hand corner of expression 126:

$$\mathbf{Q}_{\mathcal{E}\mathcal{E}} = \begin{bmatrix} -(\alpha + k_{+B}x_B) & k_{+B}x_B \\ k_{-B} & -k_{-B} \end{bmatrix} \quad (133)$$

Notice that equation 132 is analogous to 129, although it is rather simpler because it involves a smaller matrix. The upper left-hand element of $\mathbf{P}_{\mathcal{E}\mathcal{E}}(t)$ is $P'_{11}(t)$, which has already been derived in equation 52. In general, the elements of $\mathbf{P}_{\mathcal{E}\mathcal{E}}(t)$ can be expressed as the sum of $k_{\mathcal{E}}$ exponential terms; the rate constants for these terms (e.g., those given in equation 53 for simple channel block) are given by the eigenvalues of $-\mathbf{Q}_{\mathcal{E}\mathcal{E}}$ (which are $k_{\mathcal{E}}$ in number, not $k_{\mathcal{E}} - 1$, because $\mathbf{Q}_{\mathcal{E}\mathcal{E}}$, unlike \mathbf{Q} , is not singular).

13.3. The Open-Time and Shut-Time Distributions

A similar procedure can be followed for any other specified subset of states. The result will always involve a sum of exponential terms, the number of terms being equal to the number of states. For example, let us denote the set of open states as \mathcal{A} ; this would contain state 1 only for the simple mechanisms in equations 1 and 59, but it would contain states 1 and 2 for the more complex mechanism in equation 110. Again, we can define the subsection

of \mathbf{Q} that concerns transitions within \mathcal{A} states; for the mechanism in equation 110, this consists of the top left-hand 2×2 section of matrix 127:

$$\mathbf{Q}_{\mathcal{A}\mathcal{A}} = \begin{bmatrix} -(\alpha_1 + k_{+2}^* x_A) & k_{+2}^* x_A \\ 2k_{-2}^* & -(\alpha_2 + 2k_{-2}^*) \end{bmatrix} \quad (134)$$

whereas for the channel-block mechanism 29, with only one open state, we have simply, from equation 126:

$$\mathbf{Q}_{\mathcal{A}\mathcal{A}} = -(\alpha + k_{+B} x_B). \quad (135)$$

In general, we can write (see Colquhoun and Hawkes 1977, 1981, 1982) the distribution of open times as

$$f(t) = \phi e^{\mathbf{Q}_{\mathcal{A}\mathcal{A}} t} (-\mathbf{Q}_{\mathcal{A}\mathcal{A}}) \mathbf{u}_{\mathcal{A}} \quad (136)$$

with mean

$$m = \phi (-\mathbf{Q}_{\mathcal{A}\mathcal{A}}^{-1}) \mathbf{u}_{\mathcal{A}}$$

An alternative way to write the same thing is

$$f(t) = \phi \mathbf{G}_{\mathcal{A}\mathcal{F}}(t) \mathbf{u}_{\mathcal{F}} \quad (137)$$

where \mathcal{F} represents the set of shut states, and we define (as in equation 142 below)

$$\mathbf{G}_{\mathcal{A}\mathcal{F}}(t) = e^{\mathbf{Q}_{\mathcal{A}\mathcal{A}} t} \mathbf{Q}_{\mathcal{A}\mathcal{F}}$$

The result in equation 136 is an exact matrix analogue of the simple exponential distribution in equation 22 with $-\mathbf{Q}_{\mathcal{A}\mathcal{A}}$ replacing α . All that has been added are an initial vector ϕ , which specifies the relative probabilities of an opening starting in each of the open states, and a final vector, $\mathbf{u}_{\mathcal{A}}$, with $k_{\mathcal{A}}$ (the number of open states) unit elements. Despite the simple appearance of equation 136, it is perfectly general; it works for *any* mechanism, however complex.

In general, different classes of open times will have different distributions, determined by supplying an appropriate initial vector ϕ . For example, the distribution of all open times in a steady-state record is found by using ϕ_0 , defined in Chapter 20 (this volume, equation 42). The appropriate ϕ for open times after a jump are considered below, and cases such as the first or last opening in a burst are given by Colquhoun and Hawkes (1982). In some cases, when considering certain specified open times in the middle of a burst, the vector $\mathbf{u}_{\mathcal{A}}$ must be replaced by another vector that describes the way in which a burst ends (see Colquhoun and Hawkes, 1982).

If there is only one open state, both ϕ and $\mathbf{u}_{\mathcal{A}}$ are unity and so can be omitted, and in this case all classes of open times have the same distribution: for example, insertion of equation 135 into 136 gives the result, already derived (see Section 4.3), that the open time is described by a simple exponential distribution with mean $1/(\alpha + k_{+B} x_B)$.

More generally, equation 136 can be expressed without use of matrices as a sum of

exponential terms, the number of terms being equal to the number of open states and the rate constants being the eigenvalues of $-\mathbf{Q}_{AA}$, as described in Chapter 20 (this volume).

The distribution of shut times is exactly equivalent to that for open times given above, but we use the matrix \mathbf{Q}_{FF} instead of \mathbf{Q}_{AA} and replace \mathbf{u}_A by a vector \mathbf{u}_F containing k_F unit elements, where F denotes the set of all shut states (k_F in number). The initial ($1 \times k_F$) vector ϕ now gives the probability that a shut period starts in each of the shut states (in the steady state this would be given by ϕ_s ; see equation 50 of Chapter 20, this volume). Thus, the probability density is

$$f(t) = \phi e^{\mathbf{Q}_{FF}t} (-\mathbf{Q}_{FF}) \mathbf{u}_F \quad (138)$$

with mean

$$m = \phi (-\mathbf{Q}_{FF}^{-1}) \mathbf{u}_F \quad (139)$$

This distribution can be expressed as a sum of exponential terms, the number of terms being equal to the number of shut states, and the rate constants being the eigenvalues of $-\mathbf{Q}_{FF}$, as described in Chapter 20 (this volume).

We can now see, in matrix terms, why the distribution of all shut periods was so simple for the simple channel-block mechanism. The shut states are states 2 and 3 in this case, so \mathbf{Q}_{FF} consists of the lower right-hand 2×2 section of equation 126. The lack of intercommunication between the shut states in this mechanism is reflected by the fact that this submatrix is diagonal (elements not on the diagonal are zero); consequently, the eigenvalues of $-\mathbf{Q}_{FF}$ are simply its diagonal elements, k_{-B} and β' .

13.4. A General Approach to Bursts of Ion-Channel Openings

The analysis of bursts of openings can be approached in a way that is valid for any mechanism of the sort discussed above. The analysis given by Colquhoun and Hawkes (1982) starts by dividing the k states of the system into three subsets defined as follows: (1) open states, denoted \mathcal{A} (k_A in number), (2) short-lived shut states, denoted \mathcal{B} (k_B in number), and (3) long-lived shut states, denoted \mathcal{C} (k_C in number). The short-lived shut states (\mathcal{B}) are defined such that any sojourn in this set of states is brief enough to be deemed a gap within a burst, whereas a sojourn in \mathcal{C} would be deemed a gap between bursts. This is illustrated schematically in Fig. 20. The division into subsets is, of course, arbitrary; it is part of our hypothesis about how the observations should be interpreted. Furthermore, the division may depend on the conditions of the experiment (e.g., ligand concentrations) as well as on the mechanism itself.

Take, as an example, the agonist mechanism in equation 110. The set of open states, \mathcal{A} , is made up of states 1 and 2. For most plausible values of the rate constants, the lifetimes of shut states 3 and 4 will be short, so they constitute set \mathcal{B} . At low agonist concentration (but not otherwise), the lifetimes of the vacant state, 5, will be long, so it is the sole member of set \mathcal{C} . The transition rates for the mechanism, which are tabulated in matrix 127, can now be divided up according to this subdivision of states. For example, transition rates among open states are in the $k_A \times k_A$ matrix \mathbf{Q}_{AA} that has already been defined in equation 134.

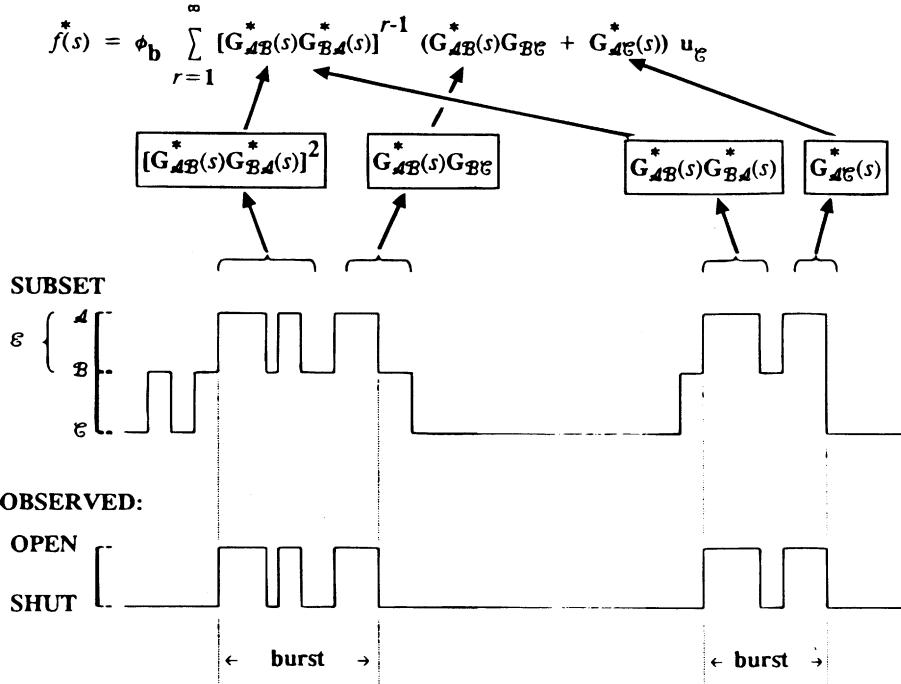


Figure 20. A more general definition of bursts of channel openings. The diagram shows two bursts, the first with $r = 3$ openings and the second with $r = 2$ openings. The two bursts shut by different routes, though this would not be visible on the experimental record; the second burst ends via a direct $\mathcal{A} \rightarrow \mathcal{C}$ transition, whereas the first gets to \mathcal{C} via \mathcal{B} . The lower part of the diagram shows the current that would be observed (if all the open states in set \mathcal{A} have the same conductance). At first, this diagram looks very like Fig. 7 (except that direct $3 \rightleftharpoons 1$ transitions were not allowed in mechanism 59). In fact, it is much more general, because the three levels in the upper diagram no longer represent three discrete states in a particular specified mechanism; they now represent three sets of states (each of which may contain any number of discrete states) that can be defined for any mechanism that results in the occurrence of channel openings in bursts. At the top, the expression for the Laplace transform of the burst length distribution (equation 149) is reproduced, and arrows show the terms in the equation that correspond to the events depicted in the diagram.

Similarly, the transition rates from \mathcal{A} states to \mathcal{B} states are in the $k_{\mathcal{A}} \times k_{\mathcal{B}}$ matrix defined, from matrix 127, as

$$\mathbf{Q}_{\mathcal{A}\mathcal{B}} = \begin{bmatrix} 0 & \alpha_1 \\ \alpha_2 & 0 \end{bmatrix} \quad (140)$$

We can define a probability density that describes the probability of staying within a particular subset, say \mathcal{A} (the open states), throughout the time from 0 to t and then leaving \mathcal{A} for a shut state in \mathcal{B} , say. For any state i that is open (in \mathcal{A}) and any state j in \mathcal{B} , this density is defined as

$$g_{ij}(t) = \lim_{\Delta t \rightarrow 0} [\text{Prob(stay within } \mathcal{A} \text{ from 0 to } t \text{ and leave } \mathcal{A} \text{ for state } j \text{ between } t \text{ and } t + \Delta t | \text{in state } i \text{ at time 0})/\Delta t] \quad (141)$$

The $k_A \times k_B$ matrix of such quantities we denote $\mathbf{G}_{AB}(t)$. It can be calculated simply as

$$\mathbf{G}_{AB}(t) = e^{\mathbf{Q}_{AA}t} \mathbf{Q}_{AB} \quad (142)$$

The $k_A \times k_A$ matrix $\mathbf{P}_{AA}(t) = e^{\mathbf{Q}_{AA}t}$ analogous to equation 132, can be expressed as the sum of k_A matrices multiplied by scalar exponential terms, as described in Chapter 20 (this volume).

13.4.1. The Number of Openings per Burst

In Section 4.6, the distribution of the number of openings per burst was derived for a simple mechanism. For more complex mechanisms, quantities like the π_{12} used there are no longer convenient. We wish to know the probabilities for transitions from, for example, A states to B states regardless of when this transition occurs. The simple quantity π_{12} is replaced by a matrix of transition probabilities, denoted simply \mathbf{G}_{AB} (the argument, t , is omitted to indicate that this now contains simple probabilities that do not depend on time). Its elements give the probabilities that the system exits from A (after any number of transitions within A states) to a particular state j in B , given that it started in state i in A . It can be calculated as

$$\mathbf{G}_{AB} = \int_0^\infty \mathbf{G}_{AB}(t) dt = -(\mathbf{Q}_{AA})^{-1} \mathbf{Q}_{AB} \quad (143)$$

which can be found directly from the relevant subsections of \mathbf{Q} defined above. Alternatively, we can take the Laplace transform of equation 142; the result for matrices is exactly analogous to that given for the simple exponential in equation 89:

$$\mathcal{L}[\mathbf{G}_{AB}(t)] = \mathbf{G}_{AB}^*(s) = (s\mathbf{I} - \mathbf{Q}_{AA})^{-1} \mathbf{Q}_{AB} \quad (144)$$

The integration in equation 143 is equivalent to setting $s = 0$ in the Laplace transform, which gives the same result as in 143. Thus, we can also define \mathbf{G}_{AB} as $\mathbf{G}_{AB}^*(0)$. Equivalent distributions involving transition from the B states to the A states are given by

$$\mathbf{G}_{BA}(t) = e^{\mathbf{Q}_{BB}t} \mathbf{Q}_{BA} \quad (145)$$

and

$$\mathbf{G}_{BA} = (-\mathbf{Q}_{BB})^{-1} \mathbf{Q}_{BA} \quad (146)$$

With the help of expressions such as this, we can, for example, write quite generally, for any mechanism, the probability that a burst contains r openings as

$$P(r) = \phi_b (\mathbf{G}_{AB} \mathbf{G}_{BA})^{r-1} (\mathbf{I} - \mathbf{G}_{AB} \mathbf{G}_{BA}) \mathbf{u}_A \quad (147)$$

with mean

$$\mathbf{m} = \phi_b (\mathbf{I} - \mathbf{G}_{AB} \mathbf{G}_{BA})^{-1} \mathbf{u}_A \quad (148)$$

These are matrix analogues of the simple expressions given in equations 36 and 38, with the matrix $\mathbf{G}_{\mathcal{A}\mathcal{B}}\mathbf{G}_{\mathcal{B}\mathcal{A}}$ describing the transitions from open to brief-shut and back to open, rather than $\pi_{12}\pi_{21}$. The only extra features that are needed (and only if there is more than one open state) are the initial vector ϕ_b , which is introduced to give the relative probabilities of a burst starting in each of the open states (see Section 6 of Chapter 20, this volume), and the usual vector of unit values, $\mathbf{u}_{\mathcal{A}}$.

As described in Chapter 20 (this volume), the distribution in equation 147 can be expressed, without use of matrices, as a mixture of geometric distributions (as in equation 58 of Chapter 19)

$$P(r) = \sum_i a_i (1 - \rho_i) \rho_i^{r-1}$$

where the ρ_i values are given by the eigenvalues of $\mathbf{G}_{\mathcal{A}\mathcal{B}}\mathbf{G}_{\mathcal{B}\mathcal{A}}$, and a_i is the area of the i th component. The number of *proper* ($\rho_i \neq 0$) geometric components is the rank of the matrix $\mathbf{G}_{\mathcal{A}\mathcal{B}}\mathbf{G}_{\mathcal{B}\mathcal{A}}$, which is at most (almost always equal to) the direct connectivity between \mathcal{A} and \mathcal{B} (see Section 10) and therefore does not exceed the smaller of $k_{\mathcal{A}}$ and $k_{\mathcal{B}}$. If the number of proper components is less than $k_{\mathcal{A}}$, there is also a component corresponding to zero eigenvalues, $\rho = 0$, which contributes to $P(1)$ but not to any other $P(r)$ (because 0^{r-1} is zero for $r > 1$, but 0^0 is taken as 1). This component is trivial in a mathematical sense, because it corresponds to the probability distribution of a random variable that can take only the value 1 but is of great practical interest because it corresponds to an excess of bursts that consist of a single opening. However, this component does not always exist even when $\mathbf{G}_{\mathcal{A}\mathcal{B}}\mathbf{G}_{\mathcal{B}\mathcal{A}}$ does have zero eigenvalues, because the area a_i attached to it may be zero. This typically happens when the connectivity between the set of open states, \mathcal{A} , and the complete set of shut states, \mathcal{F} , is the same as the direct connectivity between \mathcal{A} and \mathcal{B} ; this will be true, for example, if there is no direct connection between \mathcal{A} and \mathcal{C} , only indirect links via \mathcal{B} . Examples and further discussion of this complex point are to be found in Colquhoun and Hawkes (1987).

13.4.2. Distribution of the Burst Length

A burst starts in an open state (one of the \mathcal{A} states) and then may oscillate any number of times ($0, 1, \dots, \infty$) to the short-lived shut states (\mathcal{B} states) and back to \mathcal{A} . The probability (densities) for all possible numbers of oscillations must be added (hence the summation sign in equation 149 below). Such oscillations are illustrated in Fig. 20 for bursts with three and two openings. The burst ends at the end of the last opening, before the long-lived shut states (set \mathcal{C}) are reached. This may happen by direct transition from \mathcal{A} to \mathcal{C} (as in the second burst in Fig. 20), or it may occur *via* an intermediate sojourn in \mathcal{B} (as in the first burst in Fig. 20). In the latter case, the final sojourn in \mathcal{B} is invisible to the observer, so its duration must not be counted as part of the burst length. It is at this point that we see the great power of working with Laplace transforms. The burst length, t , consists of the sum of the lengths of many individual sojourns in different states; these may be of any length, but they add up to t . The problem is, therefore, a more complicated version of the convolution problem described in Section 9. As in Section 9, it can be solved most conveniently by multiplying the Laplace transforms of the individual distributions. Hence, we obtain a term

$\mathbf{G}_{\mathcal{B}}^*(s)\mathbf{G}_{\mathcal{B}\mathcal{A}}^*(s)$ to describe an oscillation from \mathcal{A} to \mathcal{B} and back. On the basis of this argument, we find the Laplace transform, $f^*(s)$, of the burst length distribution as

$$f^*(s) = \phi_b \sum_{r=1}^{\infty} [\mathbf{G}_{\mathcal{A}\mathcal{B}}^*(s)\mathbf{G}_{\mathcal{B}\mathcal{A}}^*(s)]^{r-1} [\mathbf{G}_{\mathcal{A}\mathcal{B}}^*(s)\mathbf{G}_{\mathcal{B}\mathcal{C}} + \mathbf{G}_{\mathcal{A}\mathcal{C}}^*(s)]\mathbf{u}_e \quad (149)$$

The final term in this describes the end of the burst. The last periods in \mathcal{A} count as part of the burst duration (as illustrated in Fig. 20); hence, the terms $\mathbf{G}_{\mathcal{A}\mathcal{B}}^*(s)$ and $\mathbf{G}_{\mathcal{A}\mathcal{C}}^*(s)$. The silent final sojourn in \mathcal{B} (see first burst in Fig. 20) is dealt with very elegantly simply by setting $s = 0$, so the last term in equation 149 contains $\mathbf{G}_{\mathcal{B}\mathcal{C}}$, i.e., $\mathbf{G}_{\mathcal{B}\mathcal{C}}(0)$, rather than $\mathbf{G}_{\mathcal{B}\mathcal{C}}^*(s)$. This notation, introduced by Colquhoun and Hawkes (1982), removes the need for the clumsier deconvolution procedures used by Colquhoun and Hawkes (1981).

The final part of the problem is to invert the Laplace transform in equation 149 to find the burst length distribution itself. This is a somewhat lengthy procedure (see Colquhoun and Hawkes, 1982), but the result is very simple. It is

$$f(t) = \phi_b [e^{\mathbf{Q}_{\mathcal{E}\mathcal{E}t}]_{\mathcal{A}\mathcal{A}} (-\mathbf{Q}_{\mathcal{A}\mathcal{A}}) e_b \quad (150)$$

where the $(k_{\mathcal{A}} \times 1)$ vector $e_b = (\mathbf{G}_{\mathcal{A}\mathcal{B}}\mathbf{G}_{\mathcal{B}\mathcal{C}} + \mathbf{G}_{\mathcal{A}\mathcal{C}})\mathbf{u}_e$ replaces the usual unit vector; it describes the paths by which the burst can end. The result in equation 150, although perfectly general, looks hardly any more complicated than the general open time distribution given in equation 136. The subscript $\mathcal{A}\mathcal{A}$ means that the calculation is done using only the upper $k_{\mathcal{A}} \times k_{\mathcal{A}}$ section of $e^{\mathbf{Q}_{\mathcal{E}\mathcal{E}t}}$ (which is a $k_{\mathcal{E}} \times k_{\mathcal{E}}$ matrix). The form of this result is intuitively appealing: it describes a sojourn in the burst states (set \mathcal{E}) that starts and ends in an open state (set \mathcal{A}). It can be expressed in scalar form, as a mixture of $k_{\mathcal{E}}$ exponentials with rates that are the eigenvalues of $-\mathbf{Q}_{\mathcal{E}\mathcal{E}}$, as described in Chapter 20 (Section 7, this volume).

13.4.3. Distribution of the Total Open Time per Burst

In Section 6.4 we discussed the fact that, under certain circumstances, if there is only one open state, then the total time for which a channel is open within a burst has an exponential distribution. Having got as far as writing equation 149 for the Laplace transform of the burst length distribution, it is very easy to obtain various related distributions, such as that for the total open time per burst. The various possible routes through the burst are described by equation 149, but now we are not interested in the time spent in the shut states, so we merely set $s = 0$ in all the $\mathbf{G}_{\mathcal{B}\mathcal{A}}^*(s)$; i.e., we replace them with $\mathbf{G}_{\mathcal{B}\mathcal{A}}$. Inversion of the result gives, again for any mechanism, a probability density with a form that is very similar to that for single open times in equation 136. It is given by

$$f(t) = \phi_b e^{\mathbf{V}_{\mathcal{A}\mathcal{A}t}} (-\mathbf{V}_{\mathcal{A}\mathcal{A}}) \mathbf{u}_A \quad (151)$$

where ϕ_b is as above. In this result, $\mathbf{V}_{\mathcal{A}\mathcal{A}}$ is a $k_{\mathcal{A}} \times k_{\mathcal{A}}$ matrix of transition rates between the set of \mathcal{A} states that takes into account the possibility of going from one to another via a sojourn in \mathcal{B} but not how long it takes to make the sojourn (because any time spent in a gap does not contribute to the total open time); thus,

$$\mathbf{V}_{\mathcal{A}\mathcal{A}} = \mathbf{Q}_{\mathcal{A}\mathcal{A}} + \mathbf{Q}_{\mathcal{A}\mathcal{B}}\mathbf{G}_{\mathcal{B}\mathcal{A}} \quad (152)$$

It follows that this distribution is also a sum of $k_{\mathcal{A}}$ exponentials whose rate constants are the eigenvalues of $-\mathbf{V}_{\mathcal{A}\mathcal{A}}$. In particular, it is a simple exponential distribution if there is only one open state.

13.5. Some Conclusions from the General Treatment

A number of general conclusions can be drawn for single-channel observations in the steady state from the analysis of Colquhoun and Hawkes (1982). For example, we can make the following statements:

1. The analysis of single-channel observations depends on submatrices of \mathbf{Q} that correspond to observable sets of states. Insofar as these are smaller than \mathbf{Q} itself, the analysis will be simpler than that of noise and relaxation experiments.
2. The number of exponential components in the distributions of various open lifetimes and of the total open time per burst should be equal to the number of open states. In practice, of course, some components may be too small to observe. In mechanisms with more than one open state, the distribution of open times will not generally be the same for all of the openings in a burst (and similarly for gaps within a burst). The distributions of durations of other intervals of interest also have distributions that are sums of exponentials. The numbers of components in these distributions are summarized in Table II.
3. In general, if a distribution contains more than one exponential component, the time constants for these components cannot be interpreted simply as the mean lifetimes of particular species, and the areas under the individual components cannot be interpreted as the number of sojourns in a particular state. Nevertheless, in particular cases, such interpretations may be approximately valid.
4. The distribution of the number of openings per burst should consist of a mixture of a number of geometric distributions; the number of components is determined by the direct connectivity of the open states, \mathcal{A} , and the short-lived shut states, \mathcal{B} . In some circumstances there may be an additional component that modifies the probability of a burst consisting of a single open time.
5. It is, for all practical purposes, not possible to analyze mechanisms such as equation 110 without the help of matrix notation. Use of this notation allows a single computer program to be written that can calculate numerically the single-channel, noise, and relaxation behavior of any specified mechanism (see Chapter 20, this volume).

Table II. Numbers of Exponential Components in Various Distributions

Type of interval	Number of components
Open times	$k_{\mathcal{A}}$
Shut times	$k_{\mathcal{B}} = k_{\mathcal{B}} + k_{\ell}$
Burst length	$k_{\ell} = k_{\mathcal{A}} + k_{\mathcal{B}}$
Total open time per burst	$k_{\mathcal{A}}$
Total shut time per burst	$k_{\mathcal{B}}$
Gaps within bursts	$k_{\mathcal{B}}$
Gaps between bursts	$k_{\bar{\mathcal{F}}} + k_{\mathcal{B}}$

13.6. Distributions following a Jump

Suppose there is a single jump of agonist concentration or voltage applied at time zero. Certain complications occur in the case where there is zero agonist concentration after the jump; there will be only a finite number of subsequent openings, and there may be none at all. We will not consider such cases here.

The basic results already given for open- and shut-time distributions still hold after a jump; the only thing that is different is the initial vector (denoted ϕ above) that describes the relative probabilities of starting in each of the open states or shut states. If, for example, the channel is shut at the moment the jump occurs, $t = 0$, then the distribution of the subsequent shut time (the *first latency*, see Section 11) is described by exactly the same expression as has already been given, but now ϕ must give the relative probabilities that the channel is in each of the shut states at $t = 0$. We denote the occupancies at time t as $\mathbf{p}(t)$ and partition this vector into the occupancies of open states $\mathbf{p}_{\mathcal{A}}(t)$ (a $1 \times k_{\mathcal{A}}$ vector) and the occupancies of shut states $\mathbf{p}_{\mathcal{F}}(t)$ (a $1 \times k_{\mathcal{F}}$ vector), as described in Chapter 20 (this volume). The relative probability of being in each shut state at $t = 0$ is therefore

$$\phi(0) = \mathbf{p}_{\mathcal{F}}(0)/\mathbf{p}_{\mathcal{F}}(0)\mathbf{u}_{\mathcal{F}}$$

where the denominator is merely the sum of the terms in the numerator, which is included to make the elements of $\phi(0)$ add up to 1. Using $\phi(0)$ in equation 138 immediately gives the distribution of first latencies. In order to use this result, we must be able to postulate appropriate values for $\mathbf{p}_{\mathcal{F}}(0)$. An example is given in Chapter 20 (this volume, Section 8). If the channel has come to equilibrium before $t = 0$, the equilibrium occupancies (under prejump conditions), calculated as in Chapter 20 (this volume, Section 3) can be used. If the channel is not at equilibrium at $t = 0$, e.g., because there was another jump just before $t = 0$, then the occupancies at $t = 0$ can be calculated as described in Chapter 20 (this volume Section 4).

More generally, when we allow for the possibility that the channel may be open at $t = 0$, we can calculate the first latency as follows. If the channel is open at time zero, the first latency is defined to be zero. Let $f_j(t)$ denote the probability density that the first latency has duration t and that when it ends, the channel enters open state j ; let $\mathbf{f}(t)$ be the row vector with elements $f_j(t)$. Then

$$\mathbf{f}(t) = p_{\mathcal{A}}(0)\delta(t) + p_{\mathcal{F}}(0)e^{\mathbf{Q}_{\mathcal{F}\mathcal{F}}' t} \mathbf{Q}_{\mathcal{F}\mathcal{A}} \quad (153)$$

where $\delta(t)$ is the Dirac delta function. The first term represents the ‘lump’ of probability at $t = 0$ that results from channels that were open at $t = 0$. The second term, which is of the form described above, gives the distributions of first latencies for channels that were shut at $t = 0$. The overall density of the first latency, $f_{sl}(t)$ say, is obtained by summing over j , so it can be written as

$$f_{sl}(t) = \mathbf{f}(t)\mathbf{u}_{\mathcal{A}} \quad (154)$$

with mean

$$m = \mathbf{p}_{\mathcal{F}}(0)(-\mathbf{Q}_{\mathcal{F}\mathcal{F}})^{-1}\mathbf{u}_{\mathcal{F}} \quad (155)$$

After this first, rather special, shut time, all subsequent open and shut times have the standard distributions given by equations 136 to 139, provided we supply the appropriate initial probability vector, ϕ . The vector of entry probabilities, for the state in which the first open time begins, is

$$\phi_{01} = \int_0^\infty \mathbf{f}(t)dt = p_A(0) + p_F(0)(-\mathbf{Q}_{FF})^{-1}\mathbf{Q}_{FA} = p_A(0) + p_F(0)\mathbf{G}_{FA} \quad (156)$$

The r th open time following the jump has a probability density given by the standard result, equation 136, but with initial vector, ϕ_{or} , given by

$$\phi_{or} = \phi_{01}(\mathbf{G}_{AF}\mathbf{G}_{FA})^{r-1} \quad r \geq 2 \quad (157)$$

and the mean for the r th open time is

$$m = \phi_{or}(-\mathbf{Q}_{AA})^{-1}\mathbf{u}_A. \quad (158)$$

Similarly, the r th shut time, for $r \geq 2$, has probability density given by equation 138 with the initial vector, ϕ , defined as

$$\phi_{sr} = \phi_{01}(\mathbf{G}_{AF}\mathbf{G}_{FA})^{r-2}\mathbf{G}_{AF} \quad r \geq 2 \quad (159)$$

The mean for the r th shut time is

$$m = \phi_{sr}(-\mathbf{Q}_{FF})^{-1}\mathbf{u}_F \quad (160)$$

The results at the end of Section 11.2 can be obtained from these formulas.

Equation 135 generalizes by use of the total probability theorem and the (strong) Markov property to

$$P_{\text{open}}(t) = \int_0^t \sum_j f_j(u) P(\text{open at } t | \text{in open state } j \text{ at time } u) du$$

i.e.,

$$P_{\text{open}}(t) = \int_0^t \mathbf{f}(u)[e^{\mathbf{Q}(t-u)}]_{A,A} \mathbf{u}_A du \quad (161)$$

where $[e^{\mathbf{Q}t}]_{A,A}$ stands for that part of the matrix $e^{\mathbf{Q}t}$ obtained by choosing only those rows and columns corresponding to open states. If there is only one open state, this is just the element $P_{11}(t)$ discussed in Section 11.3. But when there is more than one open state, we see that equation 161 does not contain the first latency distribution [i.e., $\mathbf{f}(t)\mathbf{u}_A$ from 154] as such. We thus see that there is not a simple direct relationship between the macroscopic current and the first latency distribution; rather, both can be obtained from the vector $\mathbf{f}(t)$. The macroscopic time course, $P_{\text{open}}(t)$, can be calculated as described in Chapter 20 (this volume), it will have the form of a sum of $k - 1$ exponentials with rate constants that are the eigenvalues of $-\mathbf{Q}$.

The fact that equation 161 is in the form of a convolution implies that it can be expressed simply in terms of Laplace transforms as

$$P_{\text{open}}^*(s) = \mathbf{f}^*(s)[s\mathbf{I} - \mathbf{Q}]_{\mathcal{A}\mathcal{A}}^{-1}\mathbf{u}_{\mathcal{A}} \quad (162)$$

where, by taking the Laplace transform of equation 153,

$$\mathbf{f}^*(s) = p_{\mathcal{A}}(0) + p_{\mathcal{F}}(0)(s\mathbf{I} - \mathbf{Q}_{\mathcal{F}\mathcal{F}})^{-1}\mathbf{Q}_{\mathcal{F}\mathcal{A}}. \quad (163)$$

13.7. Time Interval Omission and Maximum-Likelihood Fitting

The general nature of the problem that arises was discussed in Section 12. Here we give a brief outline of the matrix approach for general Markov mechanisms as developed in Hawkes *et al.* (1990) and Jalali and Hawkes (1992a,b), assuming a constant dead time ξ for both open and closed intervals. This follows the basic method of Ball and Sansom (1988b) of defining *e*-open times and *e*-shut times, which begin and end at time ξ after the start of the observed open and shut times (see Fig. 21), and we say that an event of type j occurs at such a point if the channel is in state j at that instant. An alternative approach (Ball *et al.*, 1991, 1993b) that concentrates on the beginnings of the observed intervals is less mathematically elegant but more physically natural, more general, and likely to be a more fruitful approach to future problems; however, in this brief outline it is simpler to use the first approach.

The key to the problem is a matrix function ${}^{\mathcal{A}}\mathbf{R}(t)$ whose ij th element (i, j being open states) is

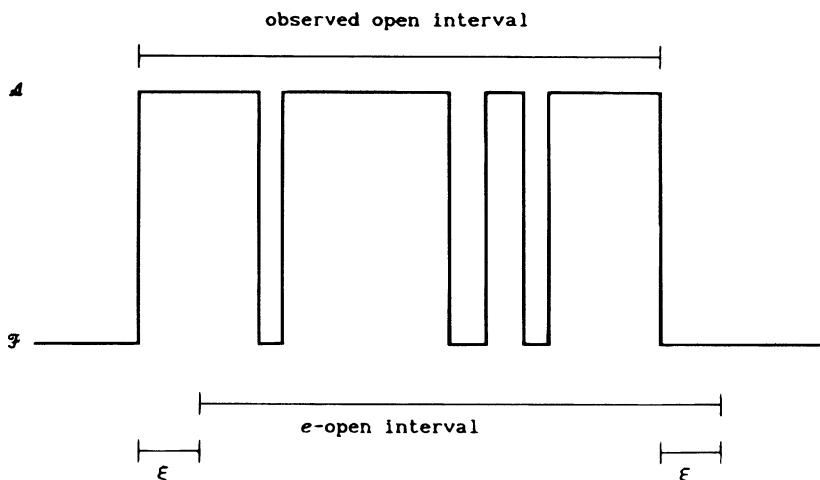


Figure 21. Illustration of the definition of an observed or apparent open interval that begins with an open time greater than ξ . An *e*-open interval has the same duration but begins time ξ after the start of the observed open interval and ends at time ξ after the start of the following observed shut interval. The events of the semi-Markov process discussed in the text occur at these points.

$${}^{\mathcal{A}}R_{ij}(t) = P[X(t) = j \text{ and no shut time is detected over } (0,t) | X(0) = i]$$

where $X(t)$ is the state of the channel at time t , and a detectable shut time is a sojourn in \mathcal{F} of duration greater than ξ . A similar function ${}^{\mathcal{F}}\mathbf{R}(t)$ is defined for shut times. Hawkes *et al.* (1990) showed how to compute ${}^{\mathcal{A}}\mathbf{R}(t)$ by a method that is quite simple for small t but becomes more complicated and numerically unstable for large t . However, Jalali and Hawkes (1992a,b) showed that it could be extremely well approximated, for all except quite small values of t , by a sum of $k_{\mathcal{A}}$ exponentials:

$${}^{\mathcal{A}}\mathbf{R}(t) \approx \sum_{i=1}^{k_{\mathcal{A}}} \mathbf{M}_i e^{-\lambda'_i t} \quad (164)$$

where the \mathbf{M}_i are $k_{\mathcal{A}} \times k_{\mathcal{A}}$ matrices, and $-\lambda'_i$ are some kind of generalized eigenvalues. They recommend using this for $t > 3\xi$ and the exact result for $t \leq 3\xi$.

Now let ${}^e\mathbf{G}_{\mathcal{AF}}(t)$ denote a semi-Markov matrix whose ij th element (i in \mathcal{A} and j in \mathcal{F}) gives the probability density of an e -open interval being of length t and the probability that it ends in shut state j , given that it began in open state i . It is given by

$${}^e\mathbf{G}_{\mathcal{AF}}(t) = {}^{\mathcal{A}}\mathbf{R}(t - \xi)\mathbf{Q}_{\mathcal{AF}} \exp(\mathbf{Q}_{\mathcal{FF}}\xi) \quad (165)$$

because, for the e -open interval to end at time t , there must be a transition from \mathcal{A} to \mathcal{F} at time $t - \xi$ (with no detectable sojourn in \mathcal{F} up to then), followed by a sojourn of at least ξ in \mathcal{F} . ${}^e\mathbf{G}_{\mathcal{AF}}(t)$ replaces the matrix function $\mathbf{G}_{\mathcal{AF}}(t)$ that occurs in the ideal ($\xi = 0$) theory. A similar function for shut times is

$${}^e\mathbf{G}_{\mathcal{FA}}(t) = {}^{\mathcal{F}}\mathbf{R}(t - \xi)\mathbf{Q}_{\mathcal{FA}} \exp(\mathbf{Q}_{\mathcal{AA}}\xi) \quad (166)$$

These functions enable us to obtain many results of interest in a form that superficially resembles those found in the ideal case.

13.7.1. Distributions of Observed Open Times and Shut Times

The probability density of observed open times is

$$f(t) = \phi_{\mathcal{A}} {}^e\mathbf{G}_{\mathcal{AF}}(t) u_{\mathcal{F}} \quad (167)$$

which may be compared with the ideal form as given in equation 137. The probability density of observed shut times is given by the similar expression:

$$f(t) = \phi_{\mathcal{F}} {}^e\mathbf{G}_{\mathcal{FA}}(t) u_{\mathcal{A}} \quad (168)$$

In these results $\phi_{\mathcal{A}}$ and $\phi_{\mathcal{F}}$ are equilibrium probability vectors for the states occupied at the start of e -open intervals or e -shut intervals, respectively. Formulas for calculating them are given by Hawkes *et al.* (1990).

The importance of result 164 is that, apart from very short times ($t < 3\xi$), the density, obtained by substituting equation 165 into expression 167, is very well approximated by a

mixture of $k_{\mathcal{A}}$ exponentials with rate constants λ'_i ; in other words, it behaves much like the true distribution of open times, having the same number of components but with modified time constants (see Section 12.3 for a numerical example). Similar results apply to equation 168, resulting in a distribution approximated by a mixture of $k_{\mathcal{F}}$ exponentials.

13.7.2. Joint Distributions of Adjacent Observed Intervals

The above results easily generalize: for example, the joint probability density of an observed shut time followed by an observed open time is given by

$$f(t_s, t_o) = \phi_{\mathcal{F}}^e \mathbf{G}_{\mathcal{F}\mathcal{A}}(t_s) {}^e\mathbf{G}_{\mathcal{A}\mathcal{F}}(t_o) u_{\mathcal{F}} \quad (169)$$

By further operations on this joint distribution, we can obtain various conditional distributions and conditional means, examples of which are given in Section 12.4. Details of these procedures are given in Srodninski (1994).

By interchanging \mathcal{A} and \mathcal{F} in the above formula we get the joint distribution of an observed open time followed by an observed shut time. For a reversible process, these two distributions are identical when dealing with *true* open and shut times. However, the method used for defining *observed* intervals is not symmetrical in time; consequently, we have found in numerical examples that these two distributions are not actually identical, though they are so close that the difference would not be detected in practice.

13.7.3. Likelihood of a Complete Record

The formula 169 is easily extended to an entire record. If, for example, we have a sequence of $2n$ intervals that starts with a shut time and ends with an open time, and if the i th pair of adjacent observed shut and open times are denoted by t_{si}, t_{oi} , then the likelihood of the entire record is given by multiplying together all the appropriate matrices. Thus, the likelihood is given by

$$\phi_{\mathcal{F}}^e \mathbf{G}_{\mathcal{F}\mathcal{A}}(t_{s1}) {}^e\mathbf{G}_{\mathcal{F}\mathcal{A}}(t_{o1}) {}^e\mathbf{G}_{\mathcal{F}\mathcal{A}}(t_{s2}) {}^e\mathbf{G}_{\mathcal{A}\mathcal{F}}(t_{o2}) \cdots {}^e\mathbf{G}_{\mathcal{F}\mathcal{A}}(t_{sn}) {}^e\mathbf{G}_{\mathcal{A}\mathcal{F}}(t_{on}) u_{\mathcal{F}} \quad (170)$$

In this expression, $\phi_{\mathcal{F}}$ is the initial vector for the first shut time; $\phi_{\mathcal{F}}^e \mathbf{G}_{\mathcal{F}\mathcal{A}}(t_{s1})$ then provides the initial vector for the first open time, and so on to the end of the record. The sequence of the openings and shuttings, and all the information on correlations contained in it, is taken into account. The likelihood defined in equation 170 can then be maximized numerically, as described in Section 12.5, to estimate the model parameters.

14. Concluding Remarks

In the first edition of this book many of the basic ideas described in this chapter were already known. The major advances since then have been in (1) the understanding of the importance of information from correlations (Section 10), (2) the development of the theory for nonstationary processes (Section 11), and (3) the development of usable theories for treating the problem of missed events (Section 12), with the concomitant ability to do direct maximum-likelihood fits of a mechanism to observed values simultaneously for several

different sets and types of data (Section 13). On the other hand, some important problems, such as the frequent problems in estimating the number of channels in a patch, remain intractable, and there has been little work on the kinetics of mechanisms that involve subconductance levels.

References

- Adams, P. R., 1976, Drug blockade of open end-plate channels, *J. Physiol.* **260**:531–552.
- Aldrich, R. W., Corey, D. P., and Stevens, C. F., 1983, A reinterpretation of mammalian sodium channel gating based on single channel recording, *Nature* **306**:436–441.
- Anderson, C. R., and Stevens, C. F., 1973, Voltage clamp analysis of acetylcholine produced end-plate current fluctuations at frog neuromuscular junction, *J. Physiol.* **235**:655–691.
- Armstrong, C. M., 1971, Interaction of tetraethylammonium ion derivatives with the potassium channels of giant axons, *J. Gen. Physiol.* **58**:413–437.
- Ball, F. G., and Sansom, M. S. P., 1988a, Single channel autocorrelation functions. The effects of time interval omission, *Biophys. J.* **53**:819–832.
- Ball, F. G., and Sansom, M. S. P., 1988b, Aggregated Markov processes incorporating time interval omission, *Adv. Appl. Prob.* **20**:546–572.
- Ball, F. G., and Sansom, M. S. P., 1989, Ion-channel mechanisms: Model identification and parameter estimation from single channel recordings, *Proc. R. Soc. Lond. [Biol.]* **236**:385–416.
- Ball, F. G., and Yeo, G. F., 1994, Numerical evaluation of observed sojourn time distributions for a single ion channel incorporating time interval omission, *Stat. Comput.* **4**:1–12.
- Ball, F. G., Kerr, C. J., Ramsey, R. L., Sansom, M. S. P., and Usherwood, P. N. R., 1988, The use of dwell time cross-correlation functions to study single-ion channel gating kinetics, *Biophys. J.* **54**:309–320.
- Ball, F. G., McGee, R., and Sansom, M. S. P., 1989, Analysis of post-perturbation gating kinetics of single ion channels, *Proc. R. Soc. Lond. [Biol.]* **236**:29–52.
- Ball, F. G., Davies, S. S., and Sansom, M. S. P., 1990, Single-channel data and missed events: Analysis of a two-state Markov model, *Proc. R. Soc. Lond. [Biol.]* **242**:61–67.
- Ball, F. G., Milne, R. K., and Yeo, G. F., 1991, Aggregated semi-Markov processes incorporating time interval omission, *Adv. Appl. Prob.* **23**:772–797.
- Ball, F. G., Milne, R. K., and Yeo, G. F., 1993a, On the exact distribution of observed open times in single ion channel models, *J. Appl. Prob.* **30**:522–537.
- Ball, F. G., Yeo, G. F., Milne, R. K., Edeson, R. O., Madsen, B. W., and Sansom, M. S. P., 1993b, Single ion channel models incorporating aggregation and time interval omission, *Biophys. J.* **64**:357–374.
- Blatz, A. L., and Magelby, K. L., 1986, Correcting single channel data for missed events, *Biophys. J.* **49**:967–980.
- Blatz, A. L., and Magleby, K. L., 1989, Adjacent interval analysis distinguishes among gating mechanisms for the fast chloride channel from rat skeletal muscle, *J. Physiol.* **410**:561–585.
- Castillo, J. del, and Katz, B., 1957, Interaction at end-plate receptors between different choline derivatives, *Proc. R. Soc. Lond. [Biol.]* **146**:369–381.
- Colquhoun, D., 1971, *Lectures on Biostatistics*, Clarendon Press, Oxford.
- Colquhoun, D., 1981, How fast do drugs work? *Trends Pharmacol. Sci.* **2**:212–217.
- Colquhoun, D., and Hawkes, A. G., 1977, Relaxation and fluctuations of membrane currents that flow through drug-operated ion channels, *Proc. R. Soc. Lond. [Biol.]* **199**:231–262.
- Colquhoun, D., and Hawkes, A. G., 1981, On the stochastic properties of single ion channels, *Proc. R. Soc. Lond. [Biol.]* **211**:205–235.
- Colquhoun, D., and Hawkes, A. G., 1982, On the stochastic properties of bursts of single ion channel openings and of clusters of bursts, *Phil. Trans. R. Soc. Lond. [Biol.]* **300**:1–59.
- Colquhoun, D., and Hawkes, A. G., 1987, A note on correlations in single ion channel records, *Proc. R. Soc. Lond. [Biol.]* **230**:15–52.
- Colquhoun, D., and Hawkes, A. G., 1990, 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, *Proc. R. Soc. Lond. [Biol.]* **240**:453–477.

- Colquhoun, D., and Hawkes, A. G., 1994, The interpretation of single channel recordings, in: *Microelectrode Techniques: The Plymouth Workshop Handbook*, 2nd ed. (D. C. Ogden, ed.) pp. 141–188, Company of Biologists, Cambridge.
- Colquhoun, D., and Ogden, D. C., 1986, States of the nicotinic acetylcholine receptor: Enumeration, characteristics and structure, in: *Nicotinic Acetylcholine Receptor, NATO ASI Series Cell Biology*, Vol. 3 (A. Maelicke, ed.) pp. 197–218, Springer, Berlin.
- Colquhoun, D., and Ogden, D. C., 1988, Activation of ion channels in the frog end-plate by high concentrations of acetylcholine, *J. Physiol.* **395**:131–159.
- Colquhoun, D., and Sakmann, B., 1981, Fluctuations in the microsecond time range of the current through single acetylcholine receptor ion channels, *Nature* **294**:464–466.
- Colquhoun, D., and Sakmann, B., 1985, Fast events in single-channel currents activated by acetylcholine and its analogues at the frog muscle end-plate, *J. Physiol.* **369**:501–557.
- Colquhoun, D., and Sheridan, R. E., 1981, The modes of action of gallamine, *Proc. R. Soc. Lond. [Biol.]* **211**:181–203.
- Colquhoun, D., Dreyer, F., and Sheridan, R. E., 1979, The actions of tubocurarine at the frog neuromuscular junction, *J. Physiol.* **293**:247–284.
- Crouzy, S. C., and Sigworth, F. J., 1990, Yet another approach to the dwell-time omission problem of single-channel analysis, *Biophys. J.* **58**:731–743.
- Cull-Candy, S. G., and Usowicz, M. M., 1987, Multiple-conductance channels activated by excitatory amino acids in cerebellar neurons, *Nature* **325**:525–528.
- Denbigh, K. G., 1951, *The Thermodynamics of the Steady State*, Methuen, London; John Wiley & Sons, New York.
- Draber, S., and Schultze, R., 1994, Correction for missed events based on a realistic model of a detector, *Biophys. J.* **66**:191–201.
- Edmonds, B., & Colquhoun, D., 1992, Rapid decay of averaged single-channel NMDA receptor activations recorded at low agonist concentration, *Proc. R. Soc. Lond. [Biol.]* **250**:279–286.
- Feller, W., 1966, *An Introduction to Probability Theory and its Applications*, 2nd ed., Vol. 2, John Wiley & Sons, New York.
- Fredkin, D. R., and Rice, J. A., 1991, *Maximum Likelihood Estimation of Ion Channel Kinetic Parameters Directly from Digitized Data*, Technical Report 338, Department of Statistics, University of California, Berkeley.
- Fredkin, D. R., Montal, M., and Rice, J. A., 1985, Identification of aggregated Markovian models: Application to the nicotinic acetylcholine receptor, in: *Procedures of the Berkeley Conference in Honor of Jerzy Neyman and Jack Kiefer*, Vol. I (L. M. Le Cam and R. A. Olshen, eds.), pp. 269–289, Wadsworth Press, Monterey, California.
- Gibb, A. J., and Colquhoun, D., 1992, Activation of NMDA receptors by L-glutamate in cells dissociated from adult rat hippocampus, *J. Physiol.* **456**:143–179.
- Gration, K. A. F., Lambert, J. J., Ramsey, R. L., Rand, R. P., and Usherwood, P. N. R., 1982, Closure of membrane channels gated by glutamate may be a two-step process, *Nature* **295**:599–601.
- Hamill, O. P., and Sakmann, B., 1981, Multiple conductance states of single acetylcholine receptor channels in embryonic muscle cells, *Nature* **294**:462–464.
- Hamill, O. P., Marty, A., Neher, E., Sakmann, B., and Sigworth, F. J., 1981, Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches, *Pflügers Arch.* **391**:85–100.
- Hawkes, A. G., Jalali, A., and Colquhoun, D., 1990, The distributions of the apparent open times and shut times in a single channel record when brief events can not be detected, *Phil. Trans. R. Soc. [A]* **332**:511–538.
- Hawkes, A. G., Jalali, A., and Colquhoun, D., 1992, Asymptotic distributions of apparent open times and shut times in a single channel record allowing for the omission of brief events, *Phil. Trans. R. Soc. [B]* **337**:383–404.
- Horn, R., 1991, Estimating the number of channels in patch recordings, *Biophys. J.* **60**:433–439.
- Horn, R., and Korn, S. J., 1989, Model selection: Reliability and bias, *Biophys. J.* **55**:379–381.
- Horn, R., and Lange, K., 1983, Estimating kinetic constants from single channel data, *Biophys. J.* **43**:207–223.
- Horn, R., and Vandenberg, C. A., 1984, Statistical properties of single sodium channels, *J. Gen. Physiol.* **84**:505–534.

- Howe, J. R., Cull-Candy, S. G., and Colquhoun, D., 1991, Currents through single glutamate-receptor channels in outside-out patches from rat cerebellar granule cells, *J. Physiol.* **432**:143–202.
- Jackson, M. B., 1985, Stochastic behaviour of a many-channel membrane system, *Biophys. J.* **47**:129–137.
- Jackson, M. B., Wong, B. S., Morris, C. E., Lecar, H. and Christian, C. N., 1983, Successive openings of the same acetylcholine receptor channels are correlated in open time, *Biophys. J.* **42**:109–114.
- Jalali, A., and Hawkes, A. G., 1992a, The distribution of apparent occupancy times in a two-state Markov process in which brief events can not be detected, *Adv. Appl. Prob.* **24**:288–301.
- Jalali, A., and Hawkes, A. G., 1992b, Generalized eigenproblems arising in aggregated Markov processes allowing for time interval omission, *Adv. Appl. Prob.*, **24**:302–321.
- Katz, B., and Miledi, R., 1970, Membrane noise produced by acetylcholine, *Nature* **226**:962–963.
- Katz, B., and Miledi, R., 1972, The statistical nature of the acetylcholine potential and its molecular components, *J. Physiol.* **224**:665–699.
- Kelly, F. P., 1979, *Reversibility and Stochastic Networks*, John Wiley & Sons, Chichester.
- Korn, J. S., and Horn, R., 1988, Statistical discrimination of fractal and Markov models of single-channel gating, *Biophys. J.* **54**:871–877.
- Labarca, P., Rice J. A., Fredkin, D. R., and Montal, M., 1985, Kinetic analysis of channel gating: application to the cholinergic receptor channel and the chloride channel from *Torpedo californica*, *Biophys. J.* **47**:469–478.
- Läuger, P., 1985, Ionic channels with conformational substates, *Biophys. J.* **47**:581–590.
- Läuger, P., 1988, Internal motions in proteins and gating kinetics of ionic channels, *Biophys. J.* **53**:877–884.
- Liebovitch, L. S., 1989, Testing fractal and Markov models of ion channel kinetics, *Biophys. J.* **55**:373–377.
- Liebovitch, L. S., Fischbarg, J., Koniarek, J. P., Todorova, I., and Wang, M., 1987, Fractal model of ion-channel kinetics, *Biochim. Biophys. Acta* **896**:173–180.
- Magleby, K. L., and Song, L., 1992, Dependency plots suggest the kinetic structure of ion channels, *Proc. R. Soc. Lond. [Biol.]* **249**:133–142.
- Magleby, K. L., and Weiss, D. S., 1990a, Estimating kinetic parameters for single channels with simulation. A general method that resolves the missed event problem and accounts for noise, *Biophys. J.* **58**:1411–1426.
- Magleby, K. L., and Weiss, D. S., 1990b, Identifying kinetic gating mechanisms for ion channels by using two-dimensional distributions of simulated dwell times, *Proc. R. Soc. Lond. [Biol.]* **241**:220–228.
- McManus, O. B., and Magleby, K. L., 1989, Kinetic time constants independent of previous single-channel activity suggest Markov gating for a large conductance Ca-activated K channel, *J. Gen. Physiol.* **94**:1037–1070.
- McManus O. B., Blatz, A. L., and Magleby, K. L., 1985, Inverse relationship of the durations of open and shut intervals for Cl and K channels, *Nature* **317**:625–627.
- McManus, O. B., Weiss, D. S., Spivak, C. E., Blatz, A. L., and Magleby, K. L., 1988, Fractal models are inadequate for the kinetics of four different ion channels, *Biophys. J.* **54**:859–870.
- Millhauser, G. L., Salpeter, E. E., and Oswald, R. E., 1988, Rate–amplitude correlation from single-channel records. A hidden structure in ion channel gating kinetics? *Biophys. J.* **54**:1165–1168.
- Milne, R. K., Yeo, G. F., Madsen, B. W., and Edeson, R. O., 1989, Estimation of single channel parameters from data subject to limited time resolution, *Biophys. J.* **55**:673–676.
- Mood, A. M., and Graybill, F. A., 1963, *Introduction to the Theory of Statistics*, 2nd ed., McGraw-Hill, New York.
- Neher, E., 1983, The charge carried by single-channel currents of rat cultured muscle cells in the presence of local anaesthetics, *J. Physiol.* **339**:663–678.
- Neher, E., and Sakmann, B., 1976, Single-channel currents recorded from membrane of denervated frog muscle fibres, *Nature* **260**:799–802.
- Neher, E., and Steinbach, J. H., 1978, Local anaesthetics transiently block currents through single acetylcholine-receptor channels, *J. Physiol.* **277**:153–176.
- Ogden, D. C. and Colquhoun, D., 1985, Ion channel block by acetylcholine, carbachol and suberyldicholine at the frog neuromuscular junction, *Proc. R. Soc. Lond. [Biol.]* **225**:329–355.
- Ogden, D. C., Siegelbaum, S. A., and Colquhoun, D., 1981, Block of acetylcholine-activated ion channels by an uncharged local anaesthetic, *Nature*, **289**:596–598.
- Olkin, I., Petkau, A. J., and Zidek, J. W., 1981, A comparison of n estimators for the binomial distribution, *J. Amer. Stat. Assoc.* **76**:637–642.
- Petracchi, D., Barbi, M., Pellegrini, M., Pellegrino, M., and Simoni, A., 1991, Use of conditional distributions in the analysis of ion channel recordings, *Eur. Biophys. J.* **20**:31–39.

- Roux, B., and Sauvé, R., 1985. A general solution to the time interval omission problem applied to single channel analysis, *Biophys. J.* **48**:149–158.
- Sakmann, B., Patlak, J., and Neher, E., 1980. Single acetylcholine-activated channels show burst-kinetics in presence of desensitizing concentrations of agonist, *Nature* **286**:71–73.
- Sansom, M. S. P., Ball, F. G., Kerry, C. J., McGee, R., Ramsey R. L., and Usherwood, P. N. R., 1989, Markov, fractal, diffusion, and related models of ion channel gating, *Biophys. J.* **56**:1229–1243.
- Sine, S. M., and Steinbach, J. H., 1986. Activation of acetylcholine receptors on clonal mammalian BC3H-1 cells by low concentrations of agonist, *J. Physiol.* **373**:129–162.
- Sine, S. M., and Steinbach, J. H., 1987. Activation of acetylcholine receptors on clonal mammalian BC3H-1 cells by high concentrations of agonist, *J. Physiol.* **385**:325–359.
- Spiegel, M. R., 1965, *Laplace Transforms*, Schaum's Outline Series, McGraw-Hill, New York.
- Srodzinski, K., 1994, *The Joint Distribution of Adjacent Open and Shut Times in Single Ion Channel Recording*, M. Phil. Thesis, University of Wales Swansea.
- Stern, P., Béhé, P., Schoepfer, R., and Colquhoun, D., 1992. Single channel properties of NMDA receptors expressed from cloned cDNAs: Comparison with native receptors, *Proc. R. Soc. Lond. [Biol.]* **250**:271–277.
- Weiss, D. S. and Magleby, K. L., 1989. Gating scheme for single GABA-activated Cl⁻ channels determined from stability plots, dwell-time distributions and adjacent-interval durations, *J. Neurosci.* **9**:1314–1324.
- Yeo, G. F., Milne, R. K., Edeson, R. O., and Madsen, B. W., 1988. Statistical inference from single channel records: Two-state Markov model with limited time resolution, *Proc. R. Soc. Lond. [Biol.]* **235**:63–94.
- Yeramian, E., Trautmann, A., and Claverie, P., 1986. Acetylcholine receptors are not functionally independent, *Biophys. J.* **50**:253–263.