

STOCHASTIC BEHAVIOR OF A MANY-CHANNEL MEMBRANE SYSTEM

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ABSTRACT A stochastic theory of channel-gating transitions is developed for a stationary system with many channels, with applications to patch-clamp single-channel experiments. Exact probability density and distribution functions for closed times, open times, and first transit times in an N -channel system are obtained in terms of N and the solutions for a one-channel system. Once N is determined, the expressions derived here can be used to analyze data records that are crowded by many channel openings and where multilevel events are common. The three-state model is treated as a specific example. Computer simulations of three-state models indicate that the equations derived here can be used to recover useful information from crowded single-channel current records. The simulations also revealed some of the limitations to the usefulness of these equations. The probability that a channel that has not opened is in a particular closed state was examined as a function of time. This analysis led to a useful limit where the distribution of unopened channels between various closed states is constant in time. This limit simplifies the mathematical treatment of closed-time probabilities, and provides a general method for the analysis of many-channel systems when channels open infrequently.

INTRODUCTION

Analysis of distributions of conductance-state lifetimes plays an important role in the interpretation of single-channel current data. Different kinetic models predict different forms of distributions; distributions are often used in quantitative determinations of rate constants.

When a single conducting state and a single nonconducting state interconvert with first-order kinetics, open times and closed times are distributed exponentially (Ehrenstein et al., 1974). The recent surge of single-channel studies, especially with patch-clamp techniques that make it possible to record single-channel currents through cell membranes (Hamill et al., 1981), has shown such simple two-state behavior to be rather exceptional. Most biologically important channels exhibit bursting behavior (Nelson and Sachs, 1979; Sakmann et al., 1980) and display open-time and closed-time distributions that are complex (Colquhoun and Sakmann, 1981, 1983; Cull-Candy and Parker, 1982; Gration et al., 1982; Dionne and Liebowitz, 1982; Liebowitz and Dionne, 1984; Jackson et al., 1982*a, b*, 1983; Auerbach and Sachs, 1983, 1984; Montal et al., 1984; Sine and Steinbach, 1984).

This more complex behavior can be explained by a number of models that invoke multiple states with the

same conductance. Colquhoun and Hawkes (1981 and 1982) have developed systematic methods for deriving probability density functions for the lifetimes of conductance levels for such models. Their treatment is formally limited to the stochastic behavior of systems with only one membrane channel. This theory is very useful in the analysis of data where channel activation is infrequent, and bursts of successive openings of a single channel are well separated from one another. However, there is always some ambiguity for a system with more than one channel: a closed time could be a time interval between a closure and opening of the same or of different channels. This ambiguity is inconsequential when channel openings are infrequent, but can be serious when data records become more crowded with many single-channel currents. In addition, when the rate of channel activation is high, records contain multilevel events produced by simultaneous openings of channels. In such cases open times cannot be uniquely determined because there is ambiguity as to which channel closes when.

There have been several reports of closed-time distributions that are well described by two exponentials (Colquhoun and Sakmann, 1981; Cull-Candy and Parker, 1982; Gration et al., 1982; Auerbach and Sachs, 1983; Jackson et al., 1983), but some investigators suggest that some closed-time data are more complex and are not adequately described by two exponential components (Colquhoun and Sakmann, 1981, 1983; Sine and Steinbach, 1984; Montal et al., 1984; Auerbach and Sachs, 1984; M. B. Jackson,

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unpublished results). Interpreting these results is very important because bursts of successive openings and closings of one channel are generally considered to be strong evidence for the existence of a special closed state that can open more rapidly than the other closed states of a channel. A rapidly opening closed state is often referred to as an activated closed state. Short closed times between openings may then be sojourns in an activated closed state, where the channel may have been activated by the binding of agonists to a receptor. Analysis of bursts of openings is therefore useful in understanding the mechanism of receptor-mediated channel activation.

In the above-cited experimental studies, conditions were controlled to provide a frequency of channel opening that is sufficiently low so as to allow the application of one-channel stochastic theory. Conditions under which channel openings are very frequent and where multilevel events are common are also easy to achieve, but the data obtained from experiments done under such conditions are more difficult to analyze. In such cases a stochastic theory for systems with many channels would be useful. Here a theory of the stochastic behavior of a stationary many-channel system is developed. These results may be useful in the analysis of single-channel current records where channel opening is frequent and multilevel events are prevalent. The theory developed here can be used to extract the true single-channel properties from data produced by the activity of many channels.

GLOSSARY

$p_i(t)$	the probability of a particular channel being in closed state i at time t subject to the constraint of not opening in the interval $(0, t)$, where initial conditions have not been specified
$r_i(t)$	$p_i(t)$ where the initial condition is that the channel closed at $t = 0$
$s_i(t)$	$p_i(t)$ where the initial condition is that the channel was closed at $t = 0$, and underwent a closing transition any time before or at $t = 0$
$g_N(t)$	the closed-time probability density for a system with N channels
$G_N(t)$	the tail distribution of the closed-time probability, i.e., the probability of a closed time being t or longer, $G_N(t) = \int_{x=t}^{\infty} g_N(x) dx$
$q_N(t)$	the first transit time probability density for an N -channel system
$Q_N(t)$	the tail distribution of the first-transit time probability, i.e., the probability of a first-transit time being t or longer, $Q_N(t) = \int_{x=t}^{\infty} q_N(x) dx$
$h(t)$	the single-channel open-time probability density
$h_N(t)$	the open-time probability density for an N -channel system where only isolated single open events are used
λ_i	the i th eigenvalue of a transition matrix. These eigenvalues are the exponential decay constants for the basis set of exponentials that form p_i , r_i , and s_i
α_i	the rate of opening from closed state i . If closed state i cannot open, then $\alpha_i = 0$
$f_i(t)$	the fraction of channels that have not opened in the time interval $(0, t)$ and are in closed state i , $f_i(t) = p_i(t) / \sum_{i=1}^m p_i(t)$.

GENERAL THEORY

The general treatment of Colquhoun and Hawkes (1981 and 1982) for one channel with different states interconverting with first-order kinetics starts with a system of differential equations that includes terms for all of the transitions that the channel can undergo. The system of equations describes the evolution through time of the probability of being in any state. To incorporate the constraint of not opening in a time interval $(0, t)$, this system of differential equations is truncated by making the open states absorbing. This amounts to setting the closing kinetic coefficients equal to zero. The solutions of these differential equations are generally sums of one or more exponentials. These exponentials can be thought of as the elements of a basis set. The decay constants for the exponentials of the basis set are the eigenvalues of the appropriate transition matrix. Initial conditions for the probabilities of being in the various closed states at $t = 0$ are needed to define the solutions completely.

The closed-time probability density of a model with only one activated closed state capable of opening (see the three-state model below) is specified by the initial condition that the probability of being in the activated closed state is 1 and the probability of being in any other closed state is zero. When a channel has many states into which it can close, the initial conditions for the closed-time probability are determined by the distribution among the various closed states immediately after closing.

Here the probability density and distribution for first transit times will also be calculated. The first transit time is defined as the time one waits until the first opening occurs, starting from an arbitrary point in time when all channels are closed. To calculate first-transit-time probability densities, we use the same differential equations that are used to calculate closed-time probabilities, but the initial condition is the equilibrium distribution between all closed states.

In general, systems with N identical channels will be discussed. Each channel has available to it m closed states. See the Glossary for definitions of the symbols. For a one-channel system, the closed-time probability density and distribution are sums over all closed states.

$$g_1(t) = \sum_{i=1}^m \alpha_i r_i(t), \quad G_1(t) = \sum_{i=1}^m r_i(t). \quad (1a)$$

Similarly, for first-transit-times

$$q_1(t) = \sum_{i=1}^m \alpha_i s_i(t), \quad Q_1(t) = \sum_{i=1}^m s_i(t). \quad (1b)$$

The many-channel probability functions will now be expressed in terms of these one-channel functions.

There are two approaches to computing probabilities for a system with N channels. The probability density can be computed directly by summing over the probability densities for opening from all possible configurations, weighted by the probabilities of finding those configurations. Alternatively, the probability tail distribution can be computed directly by multiplying together the independent probabilities that each of the N channels does not open in the designated time interval. These procedures give identical results for all of the cases that I have analyzed, with the distribution approach being simpler. For this reason, the distribution approach is used in the derivations presented here.

Starting with the first-transit-time probability distribution $Q_N(t)$, the probability that no channel opens in the interval $(0, t)$ is the product of all the probabilities that each individual channel does not open. Since the probability that a particular channel does not open in this interval is identical with the probability that it stays closed until t or longer, we can use the one-channel distributions to obtain

$$Q_N(t) = Q_1(t)^N. \quad (2)$$

The probability density function is obtained by differentiating with respect to time and changing sign

$$q_N(t) = N q_1(t) Q_1(t)^{N-1}. \quad (3)$$

The derivation of the closed-time distribution is similar to the derivation of the first-transit-time distribution, except that one channel is different from the other $N - 1$ channels, having undergone a closure at time $t = 0$. The probability that no channels open is now the product of the probability that the channel that closed at $t = 0$ does not open multiplied by the probability that the other $N - 1$ channels that were closed at $t = 0$ do not open. Thus, instead of Eq. 2, we have

$$G_N(t) = G_1(t)Q_{N-1}(t) = G_1(t)[Q_1(t)]^{N-1}, \quad (4)$$

where $Q_{N-1}(t)$ was taken from Eq. 2. Differentiating Eq. 4 with respect to time and changing sign gives $g_N(t) = g_1(t)Q_{N-1}(t) + G_1(t)q_{N-1}(t)$ for the closed-time probability density.

According to Eq. 1, $g_1(t)$, $G_1(t)$, $q_1(t)$, and $Q_1(t)$ are different sums of the same basis set of exponentials (Colquhoun and Hawkes, 1981). From Eqs. 2-4 it can be seen that the corresponding functions for an N -channel system are products of these sums and are therefore different sums of a larger basis set of exponentials. The decay constants for the N -channel basis set are formed by adding together the decay constants of the one-channel basis set in various combinations of N .

The above equations can be made more useful with a relation between the closed-time and first-transit-time probability functions. Each closed time contains all first-transit-times shorter than or equal to itself. Thus, if one examines a data set and develops a histogram of closed times, any first-transit-time shorter than t will be counted for each closed time longer than t . Therefore, the first-transit-time probability density is proportional to the probability that a closed time is t or longer. Normalization gives

$$q_N(t) = G_N(t) \left/ \int_0^\infty G_N(x) dx \right.$$

and

$$Q_N(t) = \int_t^\infty G_N(x) dx \left/ \int_0^\infty G_N(x) dx \right. \quad (5)$$

Ordinarily, the closed-time distribution $G_N(t)$ is readily obtained from a single-channel experiment. Eqs. 2, 4, and 5 can be combined to give $G_1(t)$ in terms of $G_N(t)$

$$G_1(t) = \frac{G_N(t)}{\left[\int_t^\infty G_N(x) dx \left/ \int_0^\infty G_N(x) dx \right. \right]^{(N-1)/N}} \quad (6)$$

If a record has many simultaneous channel openings, then it is feasible to perform a binomial analysis to determine N . Eq. 6 can then be used to determine $G_1(t)$. In practice $G_N(t)$ can be either the raw data or an appropriate function determined by curve fitting. When N is large it is tempting to forego a determination of N and replace $N/(N - 1)$ in Eq. 6 with 1. The approximation is clearly valid at $t = 0$ since all quantities are 1, but for large values of t , $G_1(t)$ would approach a constant value rather than decay to 0. This is because both $Q_N(t)$ and $G_N(t)$ are sums of the same basis set of exponential functions, and when t is large, the same most slowly decaying exponential dominates in both expressions. Therefore, such an approximation cannot work no matter how large N is. The practical difficulty in replacing $N/(N - 1)$ with 1 indicates a limitation in the usefulness of Eq. 6 for large N and t , which will be illustrated below.

Open-Time Distributions

The distinction between a one-channel and an N -channel system is not as clear for open times as it is for closed times. In single-channel records with simultaneous channel openings, confusion in open-time distributions results from ambiguity in ascertaining which channel closes first after two or more channels are open simultaneously. Simply ignoring the multi-

channel currents would produce errors because longer openings are more likely to have other channel openings superimposed. Nevertheless, the selection of isolated single-level openings defines precisely an observable distribution that can be extracted from single-channel current records without ambiguity. This can then be related to the open-time distribution that would be seen in a system with only one membrane channel by the relation presented below.

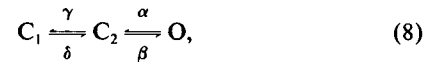
The probability density for isolated single-level channel events that are not superimposed on other channel openings is the product of the one-channel open-time probability density multiplied by the probability that no other channels open in the same time interval.

$$h'_N(t) = h(t)Q_{N-1}(t). \quad (7)$$

$h'_N(t)$ can be constructed from a crowded data record by ignoring multilevel events and used to compute $h(t)$. Here, the approximation of $Q_{N-1}(t)$ with $Q_N(t)$ when N is large may not break down as it must with Eq. 4, since the basis set of exponentials for open times is different from that for closed times.

THREE-STATE MODEL

The simple addition of an activated closed state to the two-state model gives a three-state model of the form



where C_2 is an activated closed state, C_1 is a closed state incapable of opening directly, and O is the open state. α , β , γ , and δ are the rate constants for the indicated steps. This is one of the simplest models available for describing closed-time distributions with two exponential components. When activation is caused by a receptor binding interaction, the concentration of the ligand can be incorporated into the rate γ . The procedure of Colquhoun and Hawkes (1981) is used to set up differential equations that describe the evolution through time of the probabilities, p_1 and p_2 , of being in states C_1 or C_2 . If no openings occur in the time interval $(0, t)$, we have

$$\begin{aligned} \frac{dp_1}{dt} &= -\gamma p_1 + \delta p_2 \\ \frac{dp_2}{dt} &= \gamma p_1 - (\alpha + \delta) p_2. \end{aligned} \quad (9)$$

If initial conditions are specified in terms of the initial probabilities $p_2(0) = f_2$ and $p_1(0) = 1 - f_2$, where f_2 is a constant between 0 and 1, the solutions are

$$\begin{aligned} p_2(t) &= \frac{[(\gamma + f_2\lambda_+)e^{\lambda_+t} - (\gamma + f_2\lambda_-)e^{\lambda_-t}]}{\lambda_+ - \lambda_-} \\ p_1(t) &= \frac{[(\lambda_+ + \alpha)(1 - f_2) + \delta]e^{\lambda_+t} - [(\lambda_- + \alpha)(1 - f_2) + \delta]e^{\lambda_-t}}{\lambda_+ - \lambda_-}, \end{aligned} \quad (10)$$

where the eigenvalues are

$$\lambda_{\pm} = \frac{-(\alpha + \gamma + \delta) \pm \sqrt{(\alpha + \gamma + \delta)^2 - 4\alpha\gamma}}{2}, \quad (11)$$

$f_2 = p_2(0) = 1$ is the appropriate initial condition for r_1 and r_2 since a channel that closes at $t = 0$ is in state C_2 . Since C_1 cannot open, αr_2 is the closed-time probability density for the one-channel system. s_1 and s_2 are determined by the initial condition $f_2 = p_2(0) = \gamma/(\gamma + \delta)$.

For the N -channel system Eq. 4 gives the following expression for the closed-time probability distribution

$$G_N(t) = (r_1 + r_2)(s_1 + s_2)^{N-1}.$$

Putting in the explicit forms based on Eqs. 10 for s_1 , s_2 , r_1 , and r_2 gives

$$G_N(t) = \{[(\gamma + \delta + \lambda_+)e^{\lambda_+ t} - (\gamma + \delta + \lambda_-)e^{\lambda_- t}]\} \cdot \frac{\left\{ \left[\gamma + \delta + \lambda_+ + \frac{\alpha\delta}{\gamma + \delta} \right] e^{\lambda_+ t} - \left[\gamma + \delta + \lambda_- + \frac{\alpha\delta}{\gamma + \delta} \right] e^{\lambda_- t} \right\}^{N-1}}{(\lambda_+ - \lambda_-)^N}. \quad (12)$$

When $N = 1$ this expression reduces to the one-channel case of Colquhoun and Hawkes (1981).

An approximation for Eq. 12 can be found that is applicable when the average number of channels in the activated state, $N\gamma/(\gamma + \delta)$ is low. This would be useful in applications to conditions where one-channel theory is currently applied. The type of bursting commonly seen experimentally is explained by a high α and δ relative to γ . This would suggest that an appropriate strategy for approximating Eq. 12 would be to expand it and retain lower order terms in γ . The product γN appears throughout Eq. 12 and its expansion. The validity of this approximation therefore depends on this product being small. Neglecting all but first-order terms from the expansion, we obtain

$$G_N(t) \approx \left[\frac{\delta}{(\alpha + \delta)} - \frac{(N-1)\alpha\gamma(2\delta + \alpha)}{(\alpha + \delta)^3} \right] e^{\lambda_+ t} + \left[\frac{\alpha}{(\alpha + \delta)} - \frac{(N\alpha - \alpha - 2\delta)\alpha\gamma}{\delta(\alpha + \delta)^2} \right] e^{[(N-1)\lambda_+ + \lambda_-]t} + \frac{\alpha^3\gamma(N-1)}{\delta(\alpha + \delta)^3} e^{[(N-2)\lambda_+ + 2\lambda_-]t}; \quad (13)$$

λ_+ and λ_- were retained in the exponents but elsewhere were replaced by the approximate quantities $-\alpha\gamma/(\alpha + \delta)$ and $-\alpha - \delta - \delta\gamma/(\alpha + \delta)$, respectively (small γ limit of Eq. 11). The terms that are zero order in γ can be taken to give the relatively simple expression

$$G_N(t) \approx \frac{\delta e^{\lambda_+ t}}{\alpha + \delta} + \frac{\alpha e^{[(N-1)\lambda_+ + \lambda_-]t}}{\alpha + \delta}. \quad (14)$$

Horn (1984) has derived a similar approximate expression. This calculation suggests that for a sufficiently low rate of channel activation in a many-channel system the closed-time probability distribution of the three-state model approaches a sum of two exponentials. One term is slowly

decaying and accounts for a fraction $\delta/(\alpha + \delta)$ of all closed times. The other term is rapidly decaying and accounts for a fraction $\alpha/(\alpha + \delta)$ of all closed times. The first-order corrections to the small γ limit decrease the amplitude of the two exponential components of Eq. 14 and add a third exponential component with a decay constant of $2\lambda_- + (N-2)\lambda_+$.

COMPUTER SIMULATION

The current fluctuations in a system with many channels were simulated with the aid of a computer to test the utility of the above results. Two varieties of the three-state model were considered, Eq. 8 and a variation of Eq. 8 where C_2 is replaced by a second open state. Thus, in the first case the one-channel closed-time distribution is a sum of two exponentials and the open-time distribution is a single exponential. In the second case the one-channel closed-time distribution is a single exponential and the open-time distribution is a sum of two exponentials.

The state of a system with N channels will be described by a composition (n_1, n_2, n_3) , where n_1 is the number of channels in the left-most state, n_2 the number in the middle state, and n_3 the number in the right-most state (Horn and Lange, 1983). $n_1 + n_2 + n_3 = N$. The lifetime probability of a composition decays exponentially with time with a decay rate of $\gamma n_1 + (\alpha + \delta)n_2 + \beta n_3$. A random lifetime of the composition can be generated by dividing the natural logarithm of a random number (with uniform distribution in $[0, 1]$) by this rate constant. The destination of the composition is another composition found by generating another random number and comparing it with the probabilities of the various destinations. In this way we can simulate the successive passage from composition to composition indefinitely. In all cases the simulation proceeded until 20,000 closed times were generated. The curve fits described below were obtained by least-square minimization using the Marquardt-Levenberg algorithm.

Simulation of a Two Closed-State Model

The simulated closed-time distribution of a system of 50 channels operating by this model

$$C_1 \xrightarrow{\gamma} C_2 \xrightarrow{\alpha} O$$

is plotted as curve A in Fig. 1. The rate constants are given in the figure legend. The parameters were chosen so that all 50 channels are closed only 9% of the time. At times as many as 11 channels were open simultaneously. This would be considered a very crowded data record where the application of one-channel theory (Colquhoun and Hawkes, 1981) would not be valid.

The simulated closed-time distribution overlies the curve of $G_N(t)$ computed for the same parameters using Eq. 12. A sum of two exponentials can be found that fits the simulated distribution very well, but for the same choice of

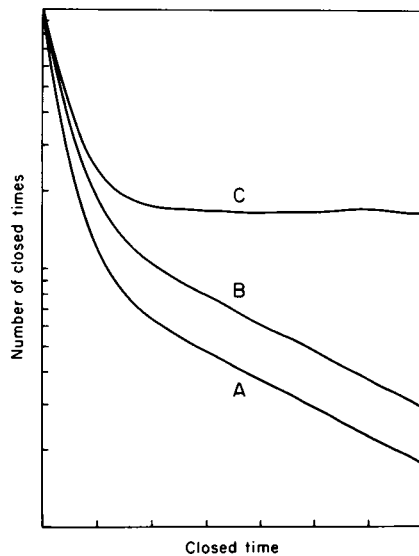


FIGURE 1 Semilogarithmic plots of closed-time distributions from a computer simulation of channels gating by a three-state mechanism $C_1 = C_2 = 0$. The simulation produced 20,000 closed times. The parameters used in this simulation were $\alpha = 0.10$, $\beta = 3 \times 10^{-2}$, $\gamma = 3 \times 10^{-4}$, $\delta = 2 \times 10^{-2}$, $N = 50$. Curve *A* is the closed-time distribution taken from this simulation. A plot of Eq. 12 for these parameters overlies curve *A*. Curve *B* is a plot of Eq. 14 for the same parameters. Curve *C* was $G_1(t)$ computed from curve *A* using a binomial estimate of N (58) and Eq. 6. A closed-time distribution obtained from a simulation with the above parameters except with $N = 1$ overlies curve *C* in the time range of this plot, but for longer times showed some slight deviations. A sum of two exponentials fit curve *C* very well. The decay constants of the fit were $\lambda_- = -0.120$ and $\lambda_+ = -2.7 \times 10^{-6}$. The computed exponential decay constants (from Eq. 11) were $\lambda_- = -0.120$ and $\lambda_+ = -2.5 \times 10^{-4}$. The slow exponential decay constant obtained from a curve fit to curve *A* was 1.23×10^{-2} . Dividing by $N = 58$ gave an estimate of $\lambda_+ = -2.1 \times 10^{-4}$. Using this value of λ_+ with λ_- and the preexponential terms from the curve fit to curve *C* to solve for the original rate constants yielded $\alpha = 0.098$, $\gamma = 2.5 \times 10^{-4}$, $\delta = 2 \times 10^{-2}$. The time scale in this figure is 0 to 140 (in arbitrary units).

parameters, Eq. 14 (curve *B* of Fig. 1) fails to reproduce the simulated closed-time distribution. With the average number of channels in the activated state $N\gamma/(\gamma + \delta) = 0.74$, appreciable deviations from Eq. 14 are evident. The deviations from Eq. 14 do not produce a discernible deviation from two exponentials, even though the first-order corrections (Eq. 13) include a third exponential component. For the parameters chosen here, the third exponential correction term is small compared with the other two correction terms. This illuminates a potential source of error. If an observed closed-time distribution obtained from a crowded single-channel current record were well fit by a sum of two exponentials, this could be misconstrued as meaning that Eq. 14 is applicable. If Eq. 14 were used to analyze such data and estimate rate constants, the estimated rate constants would not be correct.

The analysis can be improved considerably by computing the one-channel system closed-time distribution

using Eq. 6. To use Eq. 6, N must be estimated. The mean and variance in number of open channels were calculated during the simulation and used to estimate N as 58 from the predicted mean and variance of the binomial distribution. Although better methods of binomial analysis have been used to estimate N in other studies (Patlak and Horn, 1982; Sachs et al., 1982), using the variance and mean is satisfactory for the testing of these theoretical methods.

Eq. 6 was then used to compute the one-channel closed-time distribution from the simulated N -channel closed-time distribution (curve *A*) and the estimated value of N . This computation was performed numerically, point by point. The computed one-channel closed-time distribution is shown as curve *C* in Fig. 1. The computed one-channel closed-time distribution was indistinguishable from a simulated closed-time distribution for a one-channel system in the time range shown. Small but significant deviations were seen for larger t . Except for the slope of the slow exponential component, curve *C* of Fig. 1 is very similar to the true one-channel closed-time distribution. The difficulty in reconstructing $G_1(t)$ for large t and N discussed above is borne out by this simulation.

Reconstruction of $G_1(t)$ using Eq. 6 is independent of the actual model that controls channel gating. If the model is assumed, then specific model-dependent theory can be used to recover the rate constants. The fast exponential decay constant (λ_-) and the two preexponential factors are obtained from a curve fit to curve *C* of Fig. 1. Because the determination of the slow component decay constant of curve *C* has so much uncertainty, λ_+ should be determined by taking the slow exponential decay constant from curve *A* of Fig. 1 as an estimate of the quantity $N\lambda_+$, and dividing by 58, the estimated value of N . λ_+ , λ_- , and the preexponential factors can then be used to solve for α , γ , and δ using the theoretical expression predicted for the

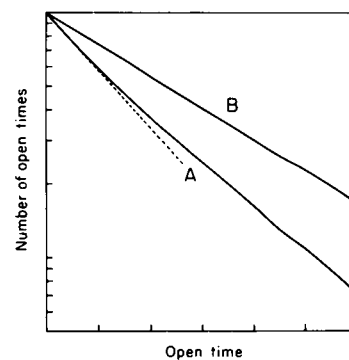


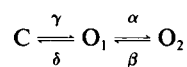
FIGURE 2 Semilogarithmic plots of open-time distributions from the same computer simulation used to generate the curves in Fig. 1. Curve *A* is the open-time distribution formed from isolated openings corresponding to $h'(t)$. A dashed line is drawn to show the deviation of curve *A* from a single exponential. $h(t)$ (curve *B*) was computed from $h'(t)$ (curve *A*) and $G_N(t)$ (curve *A* of Fig. 1) using Eqs. 2, 5, and 7. The best single-exponential fit to curve *B* yields a value of $\beta = 0.0298$ compared with the input value of 0.03. The time scale in this figure is 0 to 60 (in arbitrary units).

three-state model (see legend of Fig. 1). This represents a recovery of the original rate constants without referral to the input rate constants used in the simulation. α and δ determined in this way are remarkably close to the original values. N and γ differ by <20% from the original values. The greater uncertainty in estimating N and γ reflects the two problems of the binomial estimate of N and the reconstruction of the slow component of $G_1(t)$.

The open-time distribution from the same simulated experiment, constructed from isolated openings (9,846 isolated open events occurred in the course of the simulation), is clearly not a single exponential (curve *A* of Fig. 2). The dashed line illustrates the deviation from single-exponential behavior. Using Eq. 7 to compute $h(t)$, yields a computed one-channel open-time distribution that is a single exponential with the correct decay constant (curve *B* of Fig. 2). The variations in this decay constant using $N = 58$ or $N = 50$ are insignificant.

Simulation of a Two Open-State Model

Simulating this model of channel gating for a 50-channel system



produces an open-time distribution for isolated openings that deviates from the open-time distribution obtained from a simulation of a one-channel system with the same rate constants (compare curves *A* and *C* of Fig. 3). For 20,000 closed times there should be 20,000 open times. Only 14,562 isolated openings were seen, indicating that 5,438 open events included simultaneous openings of more

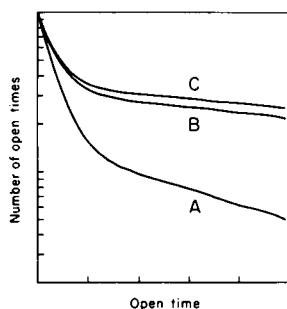


FIGURE 3 Semilogarithmic plots of open-time distributions from a computer simulation of channels, gating by a three-state mechanism $C \rightleftharpoons O_1 \rightleftharpoons O_2$. The simulation produced 20,000 closed times and 14,562 isolated one-channel open events. The parameters used in this simulation were $\alpha = 0.100$, $\beta = 10^{-2}$, $\gamma = 3 \times 10^{-4}$, $\delta = 0.20$, $N = 50$. Curve *A* is the open-time distribution of isolated single-channel openings. Curve *B* is the single-channel open-time distribution computed from curve *A* and the observed closed-time distribution using Eqs. 2, 5, and 7. Curve *C* is the open-time distribution obtained from a one-channel simulation with the same rate constants. Parameters were computed directly from a sum of two experimental curve fits to curve *C* to give $\alpha = 0.0882$, $\beta = 9.66 \times 10^{-3}$, $\delta = 0.200$. The binomial estimate of N was 32 and γ was determined from $G_N(t)$ to be 4.7×10^{-4} . The time scale in this figure is 0 to 50 (in arbitrary units).

than one channel. All channels were closed 44% of the time. Using Eqs. 2, 5, and 7, $h'(t)$ and the observed closed-time distribution were used to compute $h(t)$ (Fig. 3 curve *B*). The rate constants determined in a sum of two exponential curve fit to curve *B* can be compared with the original values used in the simulation (see Fig. 3, legend).

With higher frequencies of channel opening the simulation gave an open-time distribution for isolated openings that deviated dramatically from that obtained by simulation of a one-channel system (compare broken curve *A* with curve *D* of Fig. 4). In this simulation parameters were chosen such that all of the channels were closed 7% of the time. After simulating 20,000 closed times 12,114 isolated one-channel openings occurred.

The single-channel open-time density was computed point by point from the observed open-time and closed time distributions using Eqs. 2, 5, and 7. The distribution is shown as curve *C* of Fig. 4. This curve should be similar to

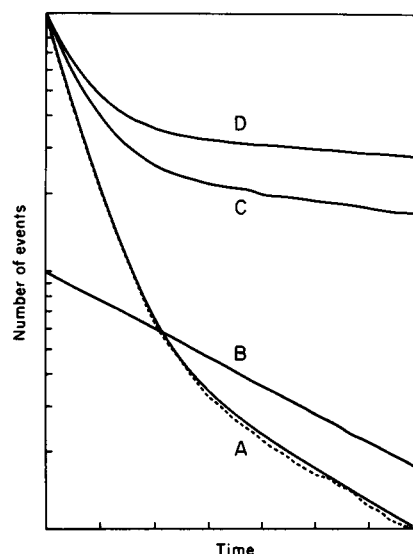


FIGURE 4 Semilogarithmic plots of open-time distributions from a computer simulation of channels gating by a three-state mechanism $C \rightleftharpoons O_1 \rightleftharpoons O_2$. The simulation produced 20,000 closed times and 12,114 isolated one-channel open events. The parameters used in this simulation were $\alpha = 0.100$, $\beta = 10^{-2}$, $\gamma = 10^{-3}$, $\delta = 0.20$, $N = 50$. The binomial estimate of N was 48. Curve *A* is the open-time distribution of isolated single-channel openings. The best-fitting sum of two exponentials was determined and differentiated with respect to time to give $h'(t)$. $Q_N(t)$ (curve *B*) was calculated from the best-fitting single exponential to the observed closed-time distribution [for a single exponential $G_N(t) = Q_N(t)$], and then raised to the 47/48 power to give $Q_{N-1}(t)$. The intercept of curve *B* was shifted down one decade to compare its slope with the long time slope of curve *A*. The similarity caused difficulty in recovering the single-channel behavior. $h(t)$ was computed using Eq. 7, and integrated to form a distribution (curve *C*). Curve *D* is an open-time distribution from a simulation using the same parameters used to generate curve *A*, except with $N = 1$. The parameters recovered from the expression for $h(t)$ computed from the observed $h'(t)$ were $\alpha = 0.0857$, $\beta = 1.35 \times 10^{-2}$, $\delta = 0.227$. These estimates were better than those obtained from a curve fit to curve *C*. From the closed-time distribution and the estimate of N , a value for γ was estimated to be 1.06×10^{-3} . The time scale in this figure is 0 to 35 (in arbitrary units).

curve D , but is not, especially for longer times. This is not because Eq. 7 is wrong, since broken curve A of Fig. 4 deviates only slightly from the predicted $h'_N(t)$ (solid curve A of Fig. 4 computed from the input parameters with Eq. 7). In fact, for the parameters used here the methods are strained to a point where a limitation appears. The reason that the method fails is that when t is large, both $h'_N(t)$ and $Q_{N-1}(t)$ decay rapidly with nearly the same rates. Their ratio, $h(t)$, which decays slowly at large t , amplifies these small variations. The small differences between the simulated and predicted $h'_N(t)$ (solid and broken curve A of Fig. 4) are within the range of statistical variations, but produce a large error in computing $h(t)$. Recovered parameters presented in the legend of Fig. 4 reflect this problem.

LOW FREQUENCY OF CHANNEL ACTIVATION

So far, the major avenue of simplification has been to approximate by letting the rate of channel activation, γ , be small (Colquhoun and Sakmann, 1981; Dionne and Liebowitz, 1982; Horn, 1984). Although this approach is successful in simplifying the three-state model, a method that can be generalized to other models would be useful. In this section an analysis of the fraction, $f_i(t)$, of channels that have not opened in the time interval $(0, t)$, and that are in closed state i , leads to approximations that can be used more generally.

Three-State Model

For the three-state model (Eq. 8), the fraction of unopened channels in state C_2 is

$$f_2(t) = \frac{p_2(t)}{p_1(t) + p_2(t)}. \quad (15)$$

The numerator and denominator of $f_2(t)$ are different linear combinations of the same two exponentials. If one of the decay constants is larger than the other ($-\lambda_- \gg -\lambda_+$), then, after a sufficient time, the rapidly decaying terms will become negligible compared with the slowly decaying terms. The remaining slow exponentials in both the numerator and denominator cancel, leaving an expression for $f_2(t)$ that is independent of time. This limiting value of $f_2(t)$ is denoted as $f_{2\infty}$. Using Eqs. 10 gives

$$f_{2\infty} = \frac{\gamma + f_2\lambda_+}{(\lambda_+ + \alpha)(1 - f_2) + \gamma + \sigma + f_2\lambda_+},$$

which simplifies to

$$f_{2\infty} = \lambda_+/\alpha. \quad (16)$$

This expression is independent of the initial state. When λ_+/α is substituted for f_2 in Eqs. 10, the fast exponential terms of p_1 and p_2 are identically zero.

Ordinarily, we can select a time point in a membrane current record, and if all we know is that all the channels

are closed, then the probability that a channel is activated is the equilibrium value $f_{2\text{eq}} = \gamma/(\gamma + \delta)$. However, as we follow the current with time, if no openings occur, then $f_2(t)$ relaxes to $f_{2\infty}$. We can then think in terms of a characteristic time during which, if no opening transitions occur, the system has relaxed to this limit. This relaxation time will be denoted as t_∞ . We can now decompose $f_{2\text{eq}}$ into two parts. More than t_∞ after the most recent closure $f_2 = f_{2\infty}$, and we are between bursts of openings. Immediately after a closure $f_2(0) = 1$, and a burst of openings is more likely to continue. $f_2(t)$ decays from 1 and passes through $f_{2\text{eq}}$ on the way to $f_{2\infty}$. $f_{2\text{eq}}$ reflects the aggregate behavior of closed times within bursts and closed time between bursts.

A system where all channels are closed most of the time is in the $f_{2\infty}$ limit most of the time. Immediately after a channel closes, it is obviously not in the $f_{2\infty}$ limit, but the other $N - 1$ channels are. As long as channel closing transitions are far apart relative to t_∞ , the initial conditions of $f_2 = f_{2\infty}$ are reasonable in determining s_1 and s_2 of Q_{N-1} . For r_1 and r_2 we still have $f_2 = 1$. With Eqs. 2 and 4 this leads to

$$G_N(t) \approx \frac{(\gamma + \delta + \lambda_+)e^{\lambda_+ t} - (\gamma + \delta + \gamma_-)e^{[\lambda_- + (N-1)\lambda_+]t}}{\lambda_+ - \lambda_-} \quad (17)$$

and

$$Q_N(t) \approx e^{\lambda_+ t} \quad (18)$$

for the closed-time and first-transit-time probability distributions for the three-state model. Eq. 17 goes to Eq. 14 for small γ .

Eqs. 17 and 18 are clearly inconsistent with Eq. 5, i.e., Eq. 17 cannot be integrated to give Eq. 18. This is because in calculating the closed-time probability the $f_{2\infty}$ limit was applied as an initial condition to all channels, except for the one that had just closed, whereas for the first-transit-time probability the $f_{2\infty}$ limit was applied to all channels.

$f_{2\infty}$ for the three-state model can be obtained by another more intuitive method as follows. After $t \gg t_\infty$, $p_1(t)$ and $p_2(t)$ decay as single exponentials with a rate of λ_+ , so

$$\frac{d[p_1(t) + p_2(t)]}{dt} = \lambda_+[p_1(t) + p_2(t)].$$

The only way for $p_1(t) + p_2(t)$ to decrease is via C_2 with rate α , so

$$\frac{d[p_1(t) + p_2(t)]}{dt} = -\alpha p_2(t).$$

This means that $\lambda_+[p_1(t) + p_2(t)] = -\alpha p_2(t)$, and Eq. 16 is recovered by applying the definition of $f_2(t)$ (Eq. 15).

General Models

These results for the three-state model can be generalized to more complicated models of channel gating. Since the probabilities of being in various closed states are sums of

exponential terms, regardless of the initial conditions, the fraction of unopened channels in a given closed state is a quotient of different linear sums of these same exponential terms. After a sufficiently long time, the slowest term will dominate and the fraction of unopened channels in each closed state will become constant; the probabilities of being in those states will then decay exponentially with a single rate, σ , which is defined as the smallest eigenvalue of the appropriate transition matrix. In cases where the transition matrix has more than one small eigenvalue of comparable magnitude, it would be difficult to realize the appropriate long time limit; it would be dangerous to assume that the fraction of unopened channels in a given state goes to a constant limit. Nevertheless, in systems where one decay constant stands out as slower than the others, a general f_∞ limit analogous to the $f_{2\infty}$ limit will be valid.

In the f_∞ limit, all p_i 's decay as single exponentials with the same rate, σ , i.e., $p_i(t) \propto f_{i\infty} e^{\sigma t}$. The first-transit-time probability distribution is then a single exponential

$$Q_N(t) \approx \left(\sum_{i=1}^m p_i \right)^N = \left(e^{\sigma t} \sum_{i=1}^m f_{i\infty} \right)^N = e^{\sigma N t}, \quad (19)$$

where $\sum_{i=1}^m f_{i\infty} = 1$. The first-transit-time probability density is then

$$q_N(t) = \sigma N e^{\sigma N t}. \quad (20)$$

Eq. 19 can be used to simplify the more general closed-time probability distribution of Eq. 4. Now, the N -channel closed-time distribution can be related to the predictions of a one-channel system by adding $(N-1)\sigma$ to each of the exponential rate constants of $G_1(t)$

$$G_N(t) = G_1(t) e^{\sigma(N-1)t}. \quad (21)$$

Thus, we see that the f_∞ limit leads to very simple approximate expressions for probability functions of an N -channel system.

It would be useful to have some guideline as to when expressions derived for the f_∞ limit are valid. The validity of Eq. 19 is necessary and sufficient for the validity of Eq. 21, since Eq. 4 is exact. Thus, $Q_{N-1}(t)$ should be examined. $Q_N(t)$ can be determined from $G_N(t)$ by Eq. 5. Here, the difference between $Q_{N-1}(t)$ and $Q_N(t)$ is unimportant for large N . The extent of its deviation from a single exponential can be determined by curve fitting to ascertain for what fraction of all closures the f_∞ limit is valid. The closed-time distribution can have very pronounced fast components, but if most times are part of a slow component, then it will be difficult to see the fast components in its integral and Eq. 19 will be valid.

N Different Channels

Consider a system with N channels each with a stable f_∞ limit. The channels are all different in terms of rate constants and can even have different gating mechanisms. Since each channel reaches a limit where the fractional

probability of being in each closed state is constant, the first-transit-time probability distribution of channel j is

$$Q_j(t) \approx e^{\sigma_j t}.$$

The first-transit-time probability distribution for the whole system is

$$\prod_{j=1}^N Q_j(t)$$

or

$$Q_N(t) \approx e^{t \sum_{j=1}^N \sigma_j}. \quad (22)$$

A single exponential of similar form was obtained by R. Horn using renewal theory for the probability density of interburst intervals (Horn, 1984). The uncertainty in assessing whether a burst has ended after the last closure is comparable to waiting t_∞ until the f_∞ limit has been reached. If rates vary too widely and some channels have stable slowly decaying activated states, such channels would reach the f_∞ limit slowly and make the application of the f_∞ limit and Eq. 22 problematic.

DISCUSSION

The extension of the stochastic theory of a one-channel system to an N -channel system is straightforward. In analyzing a system with many identical channels, the observed open-time and closed-time distributions can be used to compute the open-time and closed-time distributions for a single channel, which can then be compared with explicit model predictions as derived by Colquhoun and Hawkes (1981, 1982).

The experimental conditions in many studies involve large N and a small individual probability of opening. Under these conditions the binomial distribution goes to the Poisson distribution and information about the number of channels is lost. If conditions are altered to increase the probability of channel opening, N can be determined, but one-channel stochastic models would no longer be adequate. The theory presented here allows a determination of one-channel open-time and closed-time distributions from a crowded data record where N can also be determined.

The computer simulations demonstrate that these methods work well under ideal conditions. There are limitations that are faced as data records become increasingly crowded, especially when the kinetic mechanism is complex. It is difficult to determine systematically how the uncertainty increases as records become more crowded. This depends on many factors pertaining to the specific properties of the channels. A few useful tests help in evaluating the reliability of these methods. One test in using Eq. 7 to recover the open-time probability density, $h(t)$, is to compare the long-time decay constant of $G_N(t)$ with that of $h'(t)$, the open-time density obtained from single-channel records by neglecting multiple-channel events. If they are similar, then $h'(t)$ at long times is more a reflection of $Q_{N-1}(t)$ than $h(t)$. The reliability of Eq. 6 at

long times should be suspected if the error estimate of λ_+ or the slowest decay constant from a curve fit to the computed $G_i(t)$ is large. The success of this approach depends on how well N is known. In practice, the method of likelihood maximization will provide better estimates of N (Sachs et al., 1982; Patlak and Horn, 1982) and should improve the application of the relations presented here.

Under the less than ideal conditions that are typical in a patch-clamp experiment, many additional problems can arise. Rarely are recordings stable enough to obtain a record containing 20,000 closed times. Fewer events would lead to greater variability in the number of events in a lifetime-duration interval. This would amplify the problems described above. The methods described here would also encounter difficulty if an attempt were made to apply them to the analysis of kinetic processes that are faster than the sampling frequency or amplifier bandwidth used in an experiment.

The main value in the f_∞ limit is that it corresponds well to the limit of low frequency of channel opening. Its application to the acetylcholine receptor channel may be questionable because of the various very slow processes evident in the distribution of closed times (Colquhoun and Sakmann, 1983; Sine and Steinbach, 1984).

The commonly used approximation strategy of letting γ , the rate of activation, be small has been examined for the three-state model starting with an exact expression. Another strategy proposed here is to assume that all but one of the channels in the system have reached the f_∞ limit, where the fraction of unopened channels in a particular closed state is constant. It is difficult to establish a procedure for judging the general applicability of the small γ approximation. This will depend on the specific model and its rate constants, and on the quality of the data. In the small γ limit and the f_∞ limit, the closed-time probability distributions of the three-state model are very similar (Eqs. 14 and 17). Thus, the condition that the rate of activation is low is similar to the condition that closures of different channels rarely follow one another in quick succession.

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