CORRECTING SINGLE CHANNEL DATA FOR MISSED EVENTS

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ABSTRACT Interpretation of currents recorded from single ion channels in cellular membranes or lipid bilayers is complicated by the necessarily limited time resolution of the recording and detection systems. All intervals less than a certain duration, depending on the frequency response of the system, are not detected. Such missed events produce increases in the durations of observed open and shut intervals. In order to obtain the true kinetic scheme and rate constants underlying the observed activity, it is necessary to take into account missed events. We develop methods to correct for missed events for models with two or more states, including models with multiple open and shut states, compound states, and loops. Our methods can be used in a forward direction to predict observed distributions of open and shut intervals for a given kinetic scheme and time resolution. They can also be used in a backwards direction with iterative methods to determine rate constants consistent with the observed distributions. While a given kinetic scheme with rate constants predicts unique observed distributions of open and shut intervals, rate constants determined from observed distributions are not necessarily unique. Using these correction methods, we examine the effects of missed events for a five-state model consistent with some properties of large conductance Ca-activated K channels.

INTRODUCTION

Ion channels are widely distributed in cellular membranes. Their functions include generation of resting and action potentials, modulation of repetitive firing, initiation and regulation of secretion of neurotransmitters and hormones, and serving as receptors (Hille, 1984). Single channel recording techniques (Hamill et al., 1981; Miller, 1983) have provided a powerful new method for investigating the mechanisms of ion permeation and channel gating. The magnitude of the single channel current indicates the rate of ion flow through the channel, and step changes in the current indicate open-shut transitions of the channel. Interpretation of such single channel records is complicated, however, by the necessarily limited time resolution of the recording system. Because of limited frequency response, open and shut intervals with brief durations are not detected, leading to increases in the mean durations of the experimentally observed open and shut intervals (Sachs et al., 1982; Colquhoun and Sigworth, 1983; Magleby and Pallotta, 1983; Moczydlowski and Latorre, 1983; Neher, 1983). Even under ideal conditions of large conductance channels and minimal filtering (10 kHz), large numbers of openings and closings go undetected. Under less ideal conditions, the majority of events may be missed. In spite of this, there have been few studies on the effect of missed events, and these have been mainly concerned with two state models or their equivalent (Rickard, 1977; Sachs et al., 1982; Colquhoun and Sigworth, 1983; Magleby and Pallotta, 1983; Neher, 1983). More recently, Roux and Sauvé (1985) have presented a general formalism for the effect of missed events and describe specific examples for two- and three-state models with a single open state.

In this paper we first extend previous studies (Sachs et al., 1982; Colquhoun and Sigworth, 1983; Neher, 1983) to develop methods to correct for missed events for models with two or more states including models with multiple open and shut states. We then present a detailed analysis of the effect of missed events on single channel data for a five state model of a Ca-activated K^+ channel. Our methods can be used to correct for the effects of missed events starting with either a given kinetic scheme and rate constants or experimental data. When the starting point is a given kinetic scheme with rate constants, it is possible to predict the data that would be observed for a given time resolution. When the starting point is the experimental data, it is possible, using iterative techniques, to obtain rate constants consistent with the experimental data for a given kinetic scheme and time resolution. Some general limitations of determining underlying rate constants for experimental data, which would apply for all correction methods, are considered.

METHODS

Calculations were performed on an LSI 11/73 computer (Digital Equipment Corporation, Marlboro, MA) using either Fortran IV, or versions of

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DEC 11 Basic that we have highly modified with machine language routines.

Simulation of Distributions of Open and Shut Intervals

Computer simulation of single channel data was used to test the assumptions made when deriving equations to correct for missed events. Uncorrected single channel data were first simulated using methods similar to those of Clay and DeFelice (1983). The uncorrected simulated data were then corrected for limited time resolution to obtain the open and shut intervals that would be observed experimentally. A brief summary of the essential features of the simulation technique follows.

The mean lifetime of state i, indicated as L_i, in a given kinetic scheme is given by

$$L_i = 1/(\text{sum of rate constants leading away from state } i). \quad (1)$$

The term dwell time will be used to refer to the actual (not average) time that the channel spends in any particular state. Dwell time is a random variable (see Colquhoun and Hawkes, 1983), and is determined for each entry into a state from

$$\text{Dwell time}_i = L_i(-\ln(Rnd)), \quad (2)$$

where ln is natural log and Rnd is a random number between 0 and 1 (Cox and Miller, 1965). Starting in state i, the probability of making a transition to an adjacent state j (Prob_{ij}) is

$$\text{Prob}_{ij} = k_{ij}/(\text{sum of rate constants away from state } i), \quad (3)$$

where k_{ij} is the rate constant from state i to state j. Since the probabilities of leaving a state sum to one, the decision as to which state is entered next can be determined from cumulative probabilities and a random number between 0 and 1.

Starting in an open state, the dwell time in the state is calculated from Eq. 2, and the next state entered is determined through application of Eq. 3. The duration of the first open interval is the sum of the dwell times spent in one or more successive open states until a transition is made to a shut state. The following shut interval is then the sum of the dwell times spent in one or more successive shut states until a transition is made to an open state. The procedure is repeated until typically one million sequential open and shut intervals are generated and stored in a file. The file contains intervals that would be observed if there were no missed events.

The simulated data are then corrected for missed events assuming that a 50% amplitude threshold analysis is used to detect open and shut intervals (Sachs et al., 1982; Colquhoun and Hawkes, 1983). Because of the limited time resolution of the recording system and the almost instantaneous ($<10\ \mu$s) change in channel current (not recorded current) associated with open-shut transitions, changes in recorded current resulting from channel openings and closings typically occur with a time constant equal to that of the recording system (Hamill et al., 1981). Consequently, brief events will not reach full amplitude. With a 50% amplitude threshold analysis, events that reach less than 50% amplitude will be missed. The interval duration below which all events are missed is the dead time of the recording and analysis system, and can be measured experimentally from the pulse width that gives a half-amplitude response (Colquhoun and Sigworth, 1983).

The uncorrected simulated data are corrected for missed events by assuming all intervals less than the dead time are missed (do not cross threshold). Starting with the first open interval greater than the dead time, the durations of successive intervals are added to the open interval until a shut interval greater than the dead time is encountered. Then, starting with the shut interval, the durations of successive intervals are added to the shut interval until an open interval with duration greater than the dead time is encountered. This procedure is repeated for all the intervals in the uncorrected file. The corrected open and shut intervals are then binned into frequency histograms (number of events vs. duration) for comparison to the predicted data.

Maximum Likelihood Fitting of Distributions

Distributions of open and shut intervals were fit with sums of exponentials using the method of maximum likelihood to determine the most probable parameters for the areas and time constants (Colquhoun and Sigworth, 1983). Maximum likelihood fits were made to binned data (with increasing bin size as the duration of the intervals increased), since it was too time consuming to fit 10^5–10^6 separate data points. As long as the bin width was <5% of the duration of the midpoint of the bins, insignificant error was introduced by fitting the binned data rather than the individual observations, and the fits were thousands of times faster. The binned data used for the maximum likelihood fits had all the observed intervals in a given bin centered at the midpoint of the bin. The log of the probability of observing all the intervals in a given bin was then given by the log of the probability of observing a single interval times the number of events in the bin. The likelihood ratio test (Horn and Lange, 1983; Labarca et al., 1985) was used to determine the number of statistically significant exponentials required to fit each distribution. For plots of the simulated data, the plotted number of intervals in each bin after the first were normalized to the width of the first plotted bin by multiplying the number of intervals in each bin by the width of the first plotted bin divided by the width of each bin.

Dwell Time in Two or More Adjacent Shut or Open States

The probability that the dwell time in two or more connecting closed states (—C—C—) or two or more connecting open states (—O—O—) is less than the dead time was determined by simulating 100,000 passes through the states using Eqs. 2 and 3. The sum of all the dwell times in the states during a pass (each of the states may be entered one or more times) gives the total time for one pass. The number of passes in which the total time was less than the dead time divided by the total number of passes gives the probability that the dwell time for passage through the given sequence of states is less than the dead time.

Scaling Predicted PDFs to Simulated Data

Predicted distributions were initially calculated as probability density functions (PDF), which have an area of 1. For comparison to simulated and experimental data the predicted PDFs were then scaled by:

$$S = \frac{(\text{bin width of first plotted bin})\,(\text{number of events})}{(\text{fraction of events captured})}, \quad (4)$$

where S is the scale factor used to multiply the predicted PDF, bin width of first plotted bin is the bin size of the first plotted bin of the simulated data, number of events is the number of simulated events after correcting for dead time, fraction of events captured is given by Eq. 61 in Colquhoun and Sigworth (1983). Note that bin width and the time constants in the PDF must be expressed in the same units.

RESULTS

Missed Events for a Two-State Model

The effect of missed events will first be considered for a two-state model, and then extended to more complex models. Scheme I presents a two-state model with open state O_1, closed state C_2, and rate constants from open to shut of k_{12} and from shut to open of k_{21}.

$$C_2 \underset{k_{12}}{\overset{k_{21}}{\rightleftharpoons}} O_1$$

Scheme I

Fig. 1 *A* shows a simulated single channel current record from this model assuming infinite frequency response of the recording system. Under these idealized conditions all openings and closings of the channel are detected without distortion. Intervals that would be recorded under these idealized conditions will be referred to as true intervals. The rate constants used for this illustration, $k_{12} = 1{,}000/\text{s}$ and $k_{12} = 5{,}000/\text{s}$, give mean open and shut lifetimes of 1 and 0.2 ms, respectively. These rates were selected since the lifetime of the longest open state in kinetic schemes considered for the acetylcholine receptor channel (Colquhoun and Hawkes, 1981) and Ca-activated K channel (Magleby and Pallotta, 1983) is longer than the lifetime of the adjacent shut state.

Fig. 1 *B* shows the single channel current that would be recorded from Scheme I under conditions of limited time resolution, as would be the case for a patch clamp experiment with low pass filtering of 1.9 kHz (−3 dB). Fig. 1 *C* shows the intervals that would be measured from the currents in Fig. 1 *B* using 50% threshold detection for channel opening and closing (dashed line in *B*). The intervals in Fig. 1 *C* will be referred to as the observed intervals, as these are the ones that would be detected experimentally.

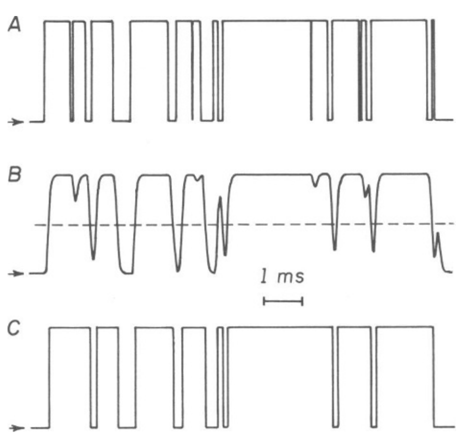


FIGURE 1 Effect of filtering and 50% threshold detection on single channel currents from a two state kinetic model. Arrows indicate closed channel current level. (*A*) True events represented as an idealized single channel current record as it would appear in the absence of any filtering. Open and shut intervals were simulated from the two state model described by Scheme I with rate constants: $k_{12} = 1{,}000/\text{s}$, $k_{21} = 5000/\text{s}$. (*B*) Currents that would actually be observed for the channel activity in *A* for analog low-pass filtering sufficient to produce a dead time (see Methods) of 0.1 ms (Ithaco model 4302 24 dB/octave, pulse mode, dial setting of 3 kHz gives −3 dB point of 1.9 kHz.) Dashed line represents the 50% current amplitude threshold used to detect channel opening and closing to construct trace *C*. The slight shift to the right from *A* is induced by the delay in the filter. (*C*) Observed events reconstructed from the filtered current in *B* using a 50% threshold detection technique to capture open and shut events. Four shut intervals and one open interval go undetected, producing increases in observed open and shut intervals when compared to the true intervals in *A*.

In this example, all intervals with durations <0.1 ms did not cross the threshold for detection, and were therefore missed. Thus, the dead time of the recording and detection system is 0.1 ms (see Methods). Fig. 1 shows that undetected intervals increase the observed durations of open and shut intervals (Fig. 1 *C*) when compared to the true durations of the intervals generated by the channel (Fig. 1 *A*).

Since the dwell times in a state are exponentially distributed (Colquhoun and Hawkes, 1983), the fraction of true shut intervals less than the dead time, $F_{\text{miss}(2)}$, is

$$F_{\text{miss}(2)} = 1 - \exp(-D/L_2), \tag{5}$$

where D is the dead time and L_2 is the mean lifetime of state 2, given by Eq. 1. The fraction of true shut intervals greater than the dead time, $F_{\text{cap}(2)}$, is

$$F_{\text{cap}(2)} = 1 - F_{\text{miss}(2)} = \exp(-D/L_2). \tag{6}$$

The fraction of true open intervals less than the dead time, $F_{\text{miss}(1)}$, and greater than the dead time, $F_{\text{cap}(1)}$, can be obtained by substituting state 1 for state 2 in Eqs. 5 and 6.

True intervals greater than the dead time will be referred to as captured intervals and those less than the dead time as missed intervals. Fig. 2 plots the captured and missed true open and shut intervals for Scheme I. Since the 0.1 ms dead time is significant compared to the 0.2 ms lifetime of the shut state, the fraction of missed true shut intervals (0.393) is appreciable, with only 0.607 of the true shut intervals captured. Fewer true open intervals are missed (0.095) and more captured (0.905), when compared with shut intervals, because of the longer lifetime of the open state.

The mean duration of all missed true shut intervals, $T_{\text{miss}(2)}$, is (Colquhoun and Sigworth, 1983; Neher, 1983)

$$T_{\text{miss}(2)} = \frac{L_2 - (L_2 + D)\exp(-D/L_2)}{1 - \exp(-D/L_2)}. \tag{7}$$

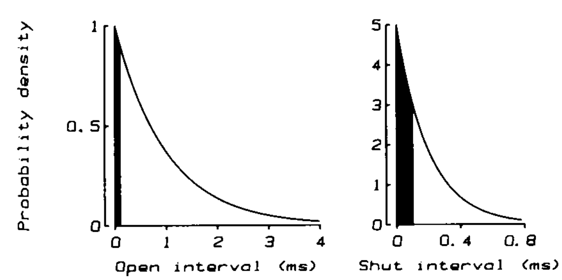


FIGURE 2 Plots of true open and shut intervals greater than the dead time (open area, captured intervals) and less than the dead time (shaded area, missed intervals) for Scheme I with a dead time of 0.1 ms, $k_{12} = 1{,}000\ \text{s}^{-1}$, and $k_{21} = 5{,}000\ \text{s}^{-1}$. Note differences in time axis for open and shut distributions.

Substituting state 1 for state 2 in Eq. 7 gives $T_{\mathrm{miss}(1)}$, the mean duration of all missed true open intervals. For the example in Figs. 1 and 2 with $L_2 = 0.2$ ms, $L_1 = 1$ ms and $D = 0.1$ ms, $T_{\mathrm{miss}(2)} = 0.0458$ ms and $T_{\mathrm{miss}(1)} = 0.0492$ ms.

Mean Duration and Time Constant of Observed Intervals

The mean duration of observed open intervals, $L_{\mathrm{obs}(1)}$, is defined by

$$L_{\mathrm{obs}(1)} = \frac{\text{total observed open time}}{\text{number of observed openings}}. \quad (8)$$

From Fig. 1 it can be seen that missed true shut intervals that occur when the current level is above the 50% threshold detection level contribute to observed open time. It can also be seen that missed true open intervals that occur when the current level is below threshold do not contribute to observed open time. Thus,

$$\begin{aligned}&\text{Total observed open time}\\ &\quad = \text{total duration of all true open intervals}\\ &\qquad + \text{total duration of missed true}\\ &\qquad\quad \text{shut intervals above threshold}\\ &\qquad - \text{total duration of missed true}\\ &\qquad\quad \text{open intervals below threshold.} \end{aligned} \quad (9)$$

From Eqs. 8 and 9

$$L_{\mathrm{obs}(1)} = \frac{NL_1 + N_{\mathrm{above}(2)} T_{\mathrm{miss}(2)} - N_{\mathrm{below}(1)} T_{\mathrm{miss}(1)}}{N_{\mathrm{obs}(1)}}, \quad (10)$$

where N is the number of true open intervals, L_1 is the mean duration of all true open intervals given by the mean lifetime of state O_1, $N_{\mathrm{below}(1)}$ is the number of missed true open intervals below threshold, $N_{\mathrm{above}(2)}$ is the number of missed true shut intervals above threshold, and $N_{\mathrm{obs}(1)}$ is the number of observed open intervals.

It will be shown in a later section that the decay of the frequency of observed open intervals for durations greater than two times the dead time is well described by a single exponential. On this basis the time constant of decay for observed open intervals, $\tau_{\mathrm{obs}(1)}$, would be described by

$$\tau_{\mathrm{obs}(1)} = L_{\mathrm{obs}(1)} - D, \quad (11)$$

since the mean of all exponentially distributed observed intervals greater than D is simply $\tau + D$ (Colquhoun and Sigworth, 1983).

The number of observed openings, $N_{\mathrm{obs}(1)}$, in Eq. 10 would be equal to the sum of the probabilities of all the different ways in which observed openings can occur, times the number of true open intervals. From Fig. 1 it can be seen that an observed opening occurs every time the single channel current makes a transition from below to above the dashed threshold line. On this basis an observed opening is generated every time a true captured shut interval is followed by a true captured open interval. This is the case since captured true shut intervals are always of sufficient duration that the current is below the threshold line and captured true open intervals are always of sufficient duration that the current would rise above the threshold line. An observed opening would also be generated if the true captured shut interval and true captured open interval were separated by one or more consecutive occurrences of the interval pair 'true missed open interval-true missed shut interval'. This is the case since, following the captured true shut interval, the true missed open intervals would not be of sufficient duration to raise the current above threshold. The observed opening would occur, as above, with the captured true open interval. Thus,

$$N_{\mathrm{obs}(1)} = NF_{\mathrm{cap}(2)}F_{\mathrm{cap}(1)} \sum_{i=0}^{\infty} [F_{\mathrm{miss}(1)}]^i [F_{\mathrm{miss}(2)}]^i. \quad (12)$$

The summation represents a geometric progression such that

$$\sum_{i=0}^{\infty} [F_{\mathrm{miss}(1)}]^i [F_{\mathrm{miss}(2)}]^i = \frac{1}{1 - F_{\mathrm{miss}(1)}F_{\mathrm{miss}(2)}}. \quad (13)$$

For the example in Figs. 1 and 2, Eqs. 12 and 13 indicate that there would be 570 observed open intervals for every 1,000 true open intervals.

$N_{\mathrm{below}(1)}$, the number of missed true open intervals below threshold for substitution into Eq. 10 is calculated from the sum of the probabilities of the different ways in which open intervals below threshold can occur, times the number of true open intervals. In the interval sequence 'captured true shut interval—missed true open interval', the missed open interval occurs below threshold and does not contribute to the observed open time. For this same interval sequence followed by any number of the interval pairs 'missed true shut interval—missed true open interval' the additional missed true open intervals would also be below threshold. Thus, the number of missed true open intervals below threshold would be given by

$$N_{\mathrm{below}(1)} = NF_{\mathrm{cap}(2)}F_{\mathrm{miss}(1)} \sum_{i=0}^{\infty} [F_{\mathrm{miss}(2)}]^i [F_{\mathrm{miss}(1)}]^i. \quad (14)$$

For the example in Figs. 1 and 2, Eqs. 13 and 14 indicate that 0.060 of all true open intervals are below threshold. Since 0.095 of all true open intervals are missed, 63% of the missed true open intervals are below threshold and do not contribute to the observed open time. The 37% of the missed true open intervals that are above threshold and contribute to observed open time would occur in interval sequences such as 'captured true open interval—missed true shut interval—missed true open interval' with any number of added interval pairs 'missed true shut interval—missed true open interval'.

Missed true shut intervals above threshold would occur in interval sequences such as 'captured true open interval—missed true shut interval' followed by any added number of the interval pair 'missed true open interval—missed true shut interval'. Thus, the number of missed true shut intervals above threshold is given by

$$N_{above(2)} = NF_{cap(1)}F_{miss(2)} \sum_{i=0}^{\infty} [F_{miss(1)}]^i[F_{miss(2)}]^i. \quad (15)$$

For the example in Figs. 1 and 2, Eqs. 13 and 15 indicate that 0.370 of all true shut intervals are above threshold. Since 0.393 of all true shut intervals are missed, 94% of the missed true shut intervals are above threshold and contribute to the observed open time. This large contribution is evident in Fig. 1. The 6% of the missed true shut intervals that are below threshold would occur in interval sequences such as 'captured true shut interval—missed true open interval—missed true shut' followed by any added number of the interval pair 'missed true open interval—missed true shut interval'.

Substitution of Eqs. 7 and 11–15 into Eq. 10 yields

$$\tau_{obs(1)} = \frac{L_1 + F_{miss(2)}T_{miss(2)}}{F_{cap(2)}}. \quad (16)$$

$\tau_{obs(2)}$, the time constant of the observed shut intervals can be calculated by substituting state 2 for 1 and state 1 for 2 in Eqs. 5–16. Eqs. 7, 11, and 16 can be transformed into the correction described by Eqs. 79 and 80 in Colquhoun and Sigworth (1983).

If the duration of a missed shut interval above threshold is negligible compared with L_1, then, from Eqs. 6 and 16,

$$\tau_{obs(1)} \approx L_1/F_{cap(2)} \approx L_1 \exp(D/L_2), \quad (17)$$

which is the correction presented by Sachs et al. (1982).

Effective Rate Constants

Defining $k_{eff(12)}$ as the effective rate constant from state 1 to 2, and substituting $1/k_{eff(12)}$ for $\tau_{obs(1)}$, and $1/k_{12}$ for L_1 in Eq. 16 gives

$$F_{rate(12)} = \frac{k_{eff(12)}}{k_{12}} = \frac{F_{cap(2)}}{1 + F_{miss(2)}T_{miss(2)}/L_1}, \quad (18)$$

where $F_{rate(12)}$ is the fraction the effective rate is of the true rate from state 1 to 2.

$F_{rate(21)}$, the fraction the effective rate $k_{eff(21)}$ is of the true rate k_{21} is given by substituting state 2 for 1 and state 1 for 2 in Eq. 18.

Predicting Distributions of Open and Shut Intervals for a Two-State Model

The distributions of open and shut intervals for a two state model with no missed events are each described by a single exponential with a time constant given by the reciprocal of the rate constant away from the state. Expressing the predicted distributions as probability density functions (PDF) in which the area is normalized to 1 (see Colquhoun and Hawkes, 1983) and substituting effective rate constants (Eq. 18) to compensate for missed events gives

$$PDF_{open} = k_{eff(12)} \exp(-tk_{eff(12)}) \quad (19)$$

$$PDF_{shut} = k_{eff(21)} \exp(-tk_{eff(21)}), \quad (20)$$

where t is time.

Predicted distributions calculated with Eqs. 19 and 20 are plotted as continuous lines in Fig. 3 *A*, *B* for the example in Figs. 1 and 2 (Scheme I with $k_{12} = 1{,}000\ s^{-1}$, $k_{21} = 5{,}000\ s^{-1}$ and $D = 0.1$ ms). Simulated distributions are plotted as filled circles. The predicted distributions give an excellent description of the simulated data. That the data are reasonably well described by a single exponential over at least five time constants is also apparent from the semilogarithmic plots.

The time constants of the predicted distributions in Fig.

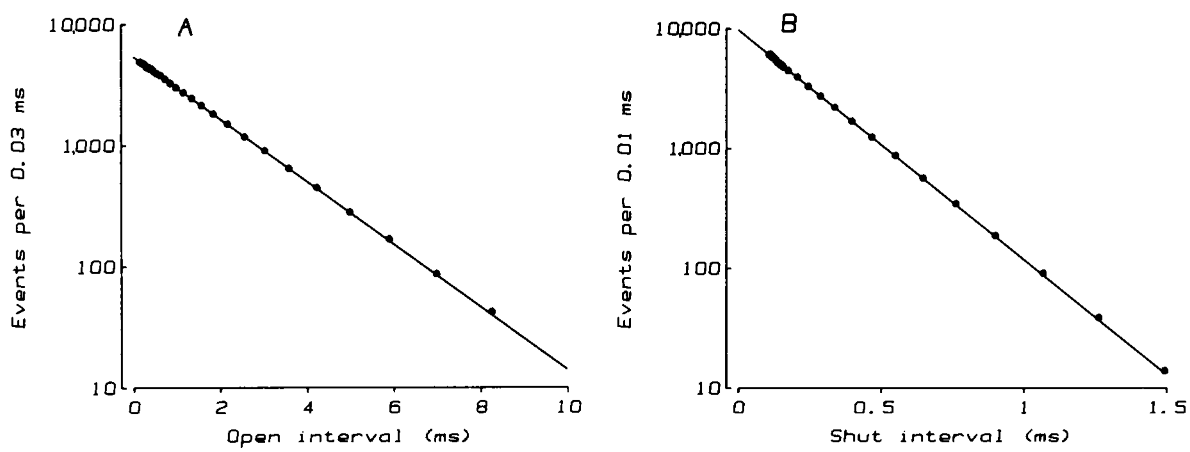


FIGURE 3 Comparison of simulated and predicted distributions of open (*A*) and shut (*B*) intervals for Scheme I with $D = 0.1$ ms, $k_{12} = 1{,}000\ s^{-1}$, and $k_{21} = 5{,}000\ s^{-1}$. The simulated data (filled circles) represents 569,798 detected events from one million open-shut transitons. The predicted distributions (lines) were calculated with effective rate constants using Eqs. 19 and 20 and scaled to the simulated data with Eq. 4. The number of observed openings calculated with Eq. 12 was 570,160, indicating an error of <0.001 in the number of observed openings in the simulated data.

3, given by the reciprocal of the effective rate constants in Eqs. 19 and 20, were 1.678 ms for the open distribution and 0.2262 ms for the shut. These estimates are almost identical (within 0.4%) to those of 1.684 ms and 0.2267 ms obtained with the most likely single exponential fit to the simulated data.

For this example, with time constants and dead time similar to those in some experimental situations, failure to account for missed events would have led to a 68% overestimation of the lifetime of the open state and a 13% overestimation of the lifetime of the shut state.

Effective rate constants and Eqs. 19 and 20 also gave excellent descriptions of the simulated data when k_{21} was increased to 10,000/s, a value also consistent with the kinetics of some channels. Predicted and simulated time constants for the open distribution were both 2.79 ms, and predicted and simulated time constants for the shut distribution were both 0.116 ms. With the 10,000/s closing rate, failure to correct for missed events would have overestimated the lifetime of the open state almost three fold, and the lifetime of the shut state by 16%.

Equivalent Kinetic Scheme

Whereas the distributions of observed open and shut intervals were well described by single exponentials in Fig. 3, the function underlying each distribution is not a true exponential (Rickard, 1977; Colquhoun and Sigworth, 1983; Roux and Sauvé, 1985). This can be seen from an equivalent scheme that incorporates missed events for a two-state model

$$\begin{array}{ccc} C_{\text{cap}(2)} & \rightleftharpoons & O_{\text{cap}(1)} \\ \updownarrow & & \updownarrow \\ X_{\text{miss}(1)} & \rightleftharpoons & X_{\text{miss}(2)}, \end{array}$$

Scheme II

where $O_{\text{cap}(1)}$ and $C_{\text{cap}(2)}$ are captured open and shut intervals, $X_{\text{miss}(2)}$ is a missed shut interval from state C_2, and $X_{\text{miss}(1)}$ a missed open interval from state O_1. $X_{\text{miss}(1)}$ and $X_{\text{miss}(2)}$ would appear as phantom states since they can form compound states with each other and with one of the captured states. These phantom states have truncated lifetimes, as indicated by the shaded areas in Fig. 2. Whether a missed event is detected as an open or shut state with 50% threshold detection (Fig. 1) depends on the previous intervals. Hence, the X designation. For example, in the interval sequence '$O_{\text{cap}(1)} - X_{\text{miss}(2)} - X_{\text{miss}(1)} - C_{\text{cap}(2)}$', the intervals $O_{\text{cap}(1)} - X_{\text{miss}(2)} - X_{\text{miss}(1)}$ would form a compound open state with both missed intervals contributing to observed open time. Interestingly, if the intervals occurred in the reverse sequence (or if the data were analyzed in the reverse direction), then the reversed interval sequence $C_{\text{cap}(2)} - X_{\text{miss}(1)} - X_{\text{miss}(2)}$ would form a compound shut state with the two missed intervals contributing to observed closed time.

Additional components arising from the phantom states resulting from missed events can become apparent when the majority of both open and shut intervals are missed (Rickard, 1977; Roux and Sauvé, 1985). Additional components, when present, are fast decaying with a time course less than about two times the dead time.

The mean observed open and shut intervals calculated with Eq. 10 or Eqs. 11 and 16 and their equivalent for the mean shut interval are exact and include all intervals from all components present. The observed time constant of decay (Eq. 16) and effective rate constants (Eq. 18) are equivalent to the best single exponential fit to the observed decays.

Determining the True Rate Constants

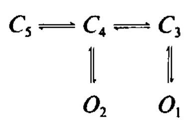
If Eqs. 16, 18, 19, and 20 adequately describe the effect of missed events, then it should be possible to determine the true rate constants from the simulated data using these equations, or, in the case of an experiment, to determine the true rate constants from the experimental data. We have used an iterative technique to solve for the true rate constants. After setting the rate constants in Scheme I to arbitrary starting values, (*a*) the predicted open and shut *PDF*s were calculated with these equations. (*b*) The probability that the simulated data was obtained from distributions described by the *PDF*s was then determined by the method of maximum likelihood. (*c*) The rate constants were changed with a pattern search maximization routine (see, for example, Colquhoun 1971) and, steps a–c repeated until the most likely rate constants were obtained. That is, the rate constants which maximized the probability that the data came from the given kinetic scheme and dead time were determined.

Even for simple two state models there was not a unique solution for the true rate constants, consistent with the findings in Colquhoun and Sigworth (1983). Two sets of rate constants with essentially identical maximum likelihood estimates could be found. The set obtained depended on the initial starting parameters. One set of most likely rate constants, 996.1/s for k_{12} and 4993/s for k_{21} was within 0.4% of the rate constants of 1,000/s and 5,000/s used to simulate the data. The other set, 12,512/s for k_{12} and 27,507/s for k_{21}, had rate constants that were markedly faster than the rate constants used to simulate the data. A decision as to which set contains the true rate constants for experimental data could be obtained by analyzing the data with different dead times. The true rate constants did not change with dead time.

Inspection of the data might also be sufficient to distinguish between the two equally likely sets of rate constants that would also be obtained for experimental data. For example, if the faster set of rate constants were the true rate constants, then over 71% of the opens and 93.6% of the shuts would be missed for a dead time of 0.1 ms. If this number of events were missed, then both the open and closed channel current could appear very noisy, due to the time average of the missed events. With the slower set of rate constants, mainly two current levels would be observed, open and shut.

Correcting for Missed Events in Models with Three or More States

Most channels are more complicated than the two state model considered above. Scheme III presents a five state model consistent with some of the properties of a Ca-activated K channel (Magleby and Pallotta, 1983) and acetylcholine receptor channel (Colquhoun and Hawkes, 1981). The rate constants are those determined by Magleby and Pallotta (1983) for the Ca-activated K channel with 0.5 μM Ca_i, and are: $k_{13} = 322/s$, $k_{31} = 3950/s$, $k_{24} = 2860/s$, $k_{42} = 120/s$, $k_{34} = 600/s$, $k_{43} = 285/s$, $k_{45} = 180/s$, and $k_{54} = 34/s$. The Ca-activated K channel is more complex than this scheme indicates (McManus and Magleby, 1985; Pallotta, 1985), but this scheme will suffice to illustrate how corrections are made for missed events in models with three or more states.



Scheme III

The general approach to obtaining the predicted distributions of open and shut intervals for this and more complicated models is the same as for the two state model. Effective rate constants are first determined and then the distributions of open and shut intervals are calculated. However, because of multiple transition pathways between open and shut states, the process is more involved.

Starting with the transition $O_1 - C_3$ in Scheme III it can be seen that there are many different open-shut-open pathways. Some of the more probable of these are

$$O_1 - C_3 - O_1 \qquad (21)$$

$$O_1 - C_3 - C_4 - O_2 \qquad (22)$$

$$O_1 - C_3 - C_4 - C_3 - O_1. \qquad (23)$$

The fraction of all shut intervals starting $O_1 - C_3$ that are missed in designated transition pathway n is

$$F_{\text{miss}(n)} = (\text{Prob pathway } n \text{ occurs}) \; (\text{Prob that the shut interval is} < D) \qquad (24)$$

where Prob is probability and D is dead time.

The probability that transition pathway $O_1 - C_3 - O_1$ occurs conditional on starting with $O_1 - C_3$ is the probability of the transition $C_3 - O_1$. Thus, from Eq. 3,

$$\text{Prob}(C_3 - O_1|C_3) = k_{31}/(k_{31} + k_{34}). \qquad (25)$$

The probability that the dwell time in C_3, $T_{\text{dwell}(3)}$, is less than the dead time is

$$\text{Prob}(T_{\text{dwell}(3)} < D) = 1 - \exp(-D/L_3), \qquad (26)$$

where L_3 is the mean lifetime of C_3 determined with Eq. 1.

Thus, starting in $O_1 - C_3$, the probability of $C_3 - O_1$ with a shut interval less than the dead time is

$$\text{Prob}(C_3 - O_1 \text{ and } T_{\text{dwell}(3)} < D|C_3) = [k_{31}/(k_{31} + k_{34})]\,[1 - \exp(-D/L_{(3)})], \qquad (27)$$

which for the rate constants in Scheme III and a dead time of 0.150 ms is $0.868 \times 0.495 = 0.429$.

In a similar manner,

$$\text{Prob}(C_3 - C_4 - O_2 \text{ and } T_{\text{dwell}(C_3-C_4)} < D|C_3) = [k_{34}/(k_{34} + k_{31})]\,[k_{42}/(k_{42} + k_{43} + k_{45})]\,[\text{Prob } T_{\text{dwell}(C_3-C_4)} < D], \qquad (28)$$

which calculates to 0.000622. The Prob $T_{\text{dwell}(C_3-C_4)} < D$ was determined by numerical convolution to be 0.023 (see Methods).

Similar calculations for all the transition pathways starting $O_1 - C_3$ indicated that $O_1 - C_3 - O_1$ (Eq. 27) was the only pathway in which a significant number of shut events were missed.

Open state O_2 closes to a shut state (C_4) that has a long mean lifetime (1.7 ms) compared to the the dead time of 0.15 ms. Consequently, few shut intervals from state C_4 are missed. The fraction of all shut intervals starting $O_2 - C_4$ that are missed in the pathway $O_2 - C_4 - O_2$ is given by

$$\text{Prob}(C_4 - O_2 \text{ and } T_{\text{dwell}(4)} < D|C_4) = [k_{42}/(k_{42} + k_{43} + k_{45})]\,[1 - \exp(-D/L_4)], \qquad (29)$$

which calculates to 0.0172. The fraction of all shut intervals starting $O_2 - C_4$ that are missed in the pathway $O_2 - C_4 - C_3 - O_1$ calculates to 0.0097. The fraction missed in other pathways starting $O_2 - C_4$ is insignificant.

Because the probability of a shut interval being less than the dead time of 0.15 ms is small for pathways that involve two or more shut states in Scheme III, few shut intervals are missed in these pathways. Consequently, for Scheme III only those pathways involving one shut state will be considered. These are described by Eqs. 27 and 29. Thus, $F_{\text{miss}(3)}$, as approximated by Eq. 27, is 0.429, giving $1 - 0.429$ or 0.571 for $F_{\text{cap}(3)}$. $F_{\text{miss}(4)}$, as approximated by Eq. 29, is 0.0172, giving $1 - 0.0172$ or 0.983 for $F_{\text{cap}(4)}$.

Using these estimates of the fraction of shut intervals captured and missed, the fractional rate constants $F_{\text{rate}(13)}$ and $F_{\text{rate}(24)}$ away from the open states are calculated using Eqs. 7 and 18. For calculation of $F_{\text{rate}(13)}$, state 3 is substituted for state 2 in Eqs. 7 and 18. For $F_{\text{rate}(24)}$ state 2 is substituted for state 1, and state 4 is substituted for state 2 in Eqs. 7 and 18.

When models contain more than one open and shut state, the calculation of the predicted distributions of open and shut intervals is no longer simple, as was the case for the two state model. Fortunately, Colquhoun and Hawkes (1981) have developed a Q-matrix method to calculate the distributions of open and shut intervals for any kinetic scheme. Their method does not correct for missed intervals, but can be used to calculate the predicted distribution for a given kinetic scheme and dead time by solving with effective rate constants. Solutions for open and shut distributions are carried out separately, using a different set of effective rate constants for each calculation.

To solve for the corrected open distribution, the rate constants for each open-shut pathway in the model are multiplied by the fractional rate given by Eq. 18 for that transition. This corrects for the missed closings due to missed shut intervals. Since each missed closing gives rise to a missed opening, the opening rate constant is also multiplied by the same fractional rate. Since both rate constants in each open-shut pair are multiplied by the same fractional rate, microscopic reversibility (or whatever apparent deviation from microscopic reversibility is present in models with closed loops (see Finkelstein and Peskin, 1984; Lauger, 1985), is preserved. For example, for Scheme III the effective rate constants used to calculate the open distribution for a dead time of 0.15 ms are

$$k_{\mathrm{eff}(13)} = F_{\mathrm{rate}(13)} \times k_{13} = 0.56538 \times 322/\mathrm{s} = 182.05/\mathrm{s} \quad (30)$$

$$k_{\mathrm{eff}(31)} = F_{\mathrm{rate}(13)} \times k_{31} = 0.56538 \times 3950/\mathrm{s} = 2233.2/\mathrm{s} \quad (31)$$

$$k_{\mathrm{eff}(24)} = F_{\mathrm{rate}(24)} \times k_{24} = 0.97920 \times 2860/\mathrm{s} = 2800.5/\mathrm{s} \quad (32)$$

$$k_{\mathrm{eff}(42)} = F_{\mathrm{rate}(24)} \times k_{42} = 0.97920 \times 120/\mathrm{s} = 117.50/\mathrm{s}. \quad (33)$$

Thus, both the forward and reverse rate constants between the open and shut states are reducing by the same fractional amount. Rate constants not directly between open and shut states remain unchanged.

The effective rate constants used to calculate the shut distribution can be calculated in a similar manner. Since the open states are not compound, $F_{\mathrm{cap}(1)}$ and $F_{\mathrm{cap}(2)}$ can be calculated directly. (For models with compound open states, the fraction of open intervals captured can be calculated using techniques similar to those described by Eqs. 21–29). To calculate $F_{\mathrm{rate}(42)}$, state 4 is substituted for state 2 in Eqs. 5–7, and state 4 is substituted for state 1 in Eq. 18. To calculate $F_{\mathrm{rate}(31)}$ state 3 is substituted for state 2 in Eqs. 5–7 and Eq. 18 and state 1 is substituted for state 2 in Eq. 18. L_3 and L_4 used in Eq. 18 are calculated with Eq. 1. The effective rate constants are

$$k_{\mathrm{eff}(31)} = F_{\mathrm{rate}(31)} \times k_{31} = 0.93788 \times 3950/\mathrm{s} = 3704.6/\mathrm{s} \quad (34)$$

$$k_{\mathrm{eff}(13)} = F_{\mathrm{rate}(31)} \times k_{13} = 0.93788 \times 322/\mathrm{s} = 302.00/\mathrm{s} \quad (35)$$

$$k_{\mathrm{eff}(42)} = F_{\mathrm{rate}(42)} \times k_{42} = 0.64203 \times 120/\mathrm{s} = 77.044/\mathrm{s} \quad (36)$$

$$k_{\mathrm{eff}(24)} = F_{\mathrm{rate}(42)} \times k_{24} = 0.64203 \times 2860/\mathrm{s} = 1836.2/\mathrm{s}. \quad (37)$$

Once again, rate constants not directly between open and shut states remain unchanged.

The predicted open and shut *PDF*s for Scheme III calculated separately with the above sets of effective rate constants using the Q-matrix method of Colquhoun and Hawkes (1981) are listed in Table I. In order to check the predicted distributions, we simulated distributions of intervals for Scheme III using the true rate constants and a dead time of 0.15 ms, and listed their best fit parameters in Table I. The areas and time constants of the predicted distributions are within 0.1–3% of those obtained from the simulated data. Parameters that would be observed if no events were missed are also presented. Missing events increased the time constants of all the components in the distributions. The greatest effect was on the open component with longest time constant, which increased from 3.11 ms for no missed events to 5.5 ms for a dead time of 0.15 ms.

Fig. 4 shows that the predicted distributions for Scheme III (continuous lines) give an excellent description of the simulated data (circles).

Using the iterative method described in previous sections, most likely true rate constants for Scheme III were determined from the simulated distributions of open and shut intervals. Similar to the two state model, several equally likely solutions for the rate constants could be found. The incorrect solutions which arose from the effect of missed events could be identified by analysis of the data with different dead times. Even after excluding incorrect solutions arising from missed events, two equally likely sets of rate constants could be obtained. The second solution had the relative lifetimes of the open states reversed. Yet, if the determined rate constants for both solutions were then used to simulate distributions, the data simulated with the different sets of determined rate constants superimposed on the data simulated with the true rate constants. This indicates that an open and shut *PDF* obtained for one condition are not necessarily sufficient to uniquely define all rate constants in models with at least three shut and two open states.

TABLE I
PREDICTED AND SIMULATED *PDF*s OF OPEN AND SHUT INTERVALS FOR SCHEME III*

	τ_1	Area_1	τ_2	Area_2	τ_3	Area_3
	ms		*ms*		*ms*	
OPEN *PDF*						
No correction	0.350	0.0601	3.11	0.940		
Predicted	0.357	0.0997	5.49	0.900		
Simulated	0.358	0.0980	5.50	0.902		
SHUT *PDF*						
No correction	0.218	0.793	1.81	0.141	44.8	0.0662
Predicted	0.230	0.801	1.96	0.130	47.0	0.0691
Simulated	0.230	0.807	1.96	0.126	47.5	0.0670

Time constants (τ) and areas for the different exponential components that sum to form the *PDF*s are presented. No correction indicates data with dead time of 0 ms that gives no missed events.
*Dead time of 0.15 ms.

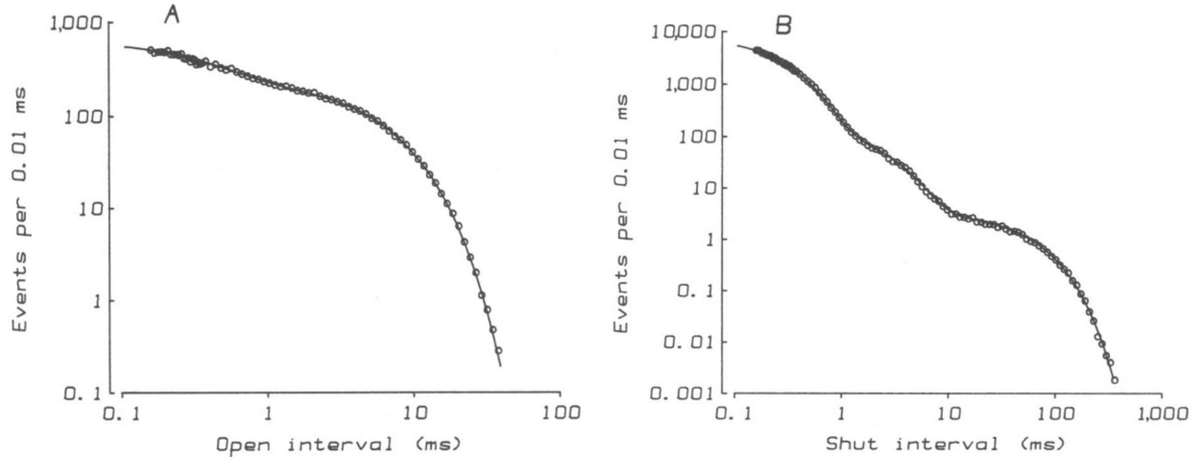


FIGURE 4 Comparison of simulated and predicted distributions of open and shut intervals for the five state model described by Scheme III. The simulated data (filled circles) represents 280,610 detected events from 500,000 open-shut transitons. The predicted distributions (lines) were calculated with effective rate constants (Eqs. 30–37) and the Q-Matrix method of Colquhoun and Hawkes (1981), and scaled to the simulated data with Eq. 4. The open intervals in (*A*) are described by the sum of two exponentials and the shut intervals in (*B*) by the sum of three (Table I). Log-log plots are used to show that the predicted distributions describe the data over the three to seven orders of magnitude of numbers of events and duration. Dead time of 0.15 ms.

The values of the 'correct set' of determined rate constants were within 1% of the values used to simulate the data, except for k_{34} which was 3.8% too slow.

This error in k_{34} could arise from transitions between open and shut states other than those described by Eqs. 27 and 29 and from the fact that the correction of the duration of missed events by Eq. 18 is only approximate for models in which the lifetime of the state associated with the effective rate constant is determined by other rate constants as well.

Comparison between Predicted and Simulated Data for Other Models

We have also compared predicted and simulated data for four- and six-state models in which the rate constants were selected so that large fractions (0.3–0.6) of both open and shut intervals were missed, leading to two to three times increases in the time constants of both open and shut distributions. In these additional models the differences between predicted and simulated distributions were typically <1–4%. When the models had loops so that there were multiple pathways to travel between some of the states, as would occur if transitions between O_1 and O_2 are added to Scheme III, similar close agreement between predicted and simulated distributions was obtained.

As was the case for Scheme III, true rate constants in models with loops could not always be uniquely determined. For some models with loops the numbers of equally likely solutions could exceed five or six. This observation is consistent with the theoretical conclusions of Fredkin et al. (1985), that a model containing cycles is not always uniquely identifiable.

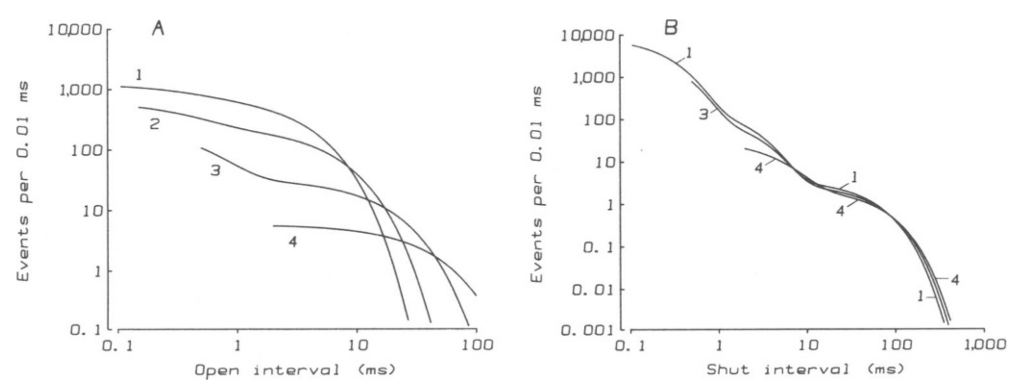


FIGURE 5 Increasing dead time reduces the number of captured events and lengthens the time constants of the exponential components of the observed open (*A*) and shut (*B*) distributions. Continuous lines are maximum likelihood fits to data simulated with Scheme III with different dead times. The dead times used were: curve 1, no dead time; curve 2, 0.15 ms; curve 3, 0.5 ms; curve 4, 2 ms. Note that the distributions are shifted down (less events detected) and to the right (time constants increased) as the dead time is increased.

Effect of Increased Dead Time

Fig. 5 plots the distributions of open and shut intervals that would be observed for Scheme III for no missed events (0 dead time), and dead times of 0.15, 0.5, and 2 ms. The distributions were obtained by fitting simulated data with the sums of exponentials. (The simulated data points were well described by the continuous lines and have not been plotted.) Increasing the dead time decreased the number of observed events. With no missed events there were 500,000 open and shut intervals. This number decreased to 280,610 (56% of true), 103,546 (21%), and 40,056 (8%) events for dead times of 0.15, 0.5, and 2 ms, respectively.

Increasing the dead time also decreased the number of exponential components. Two exponential components were required to describe the distribution of open intervals and three for the shut for dead times of 0, 0.15, and 0.5 ms. These are the numbers expected for a model with two open and three shut states, as is the case for Scheme III. When the dead time was increased to 2 ms evidence for the fastest open and shut states could no longer be detected. The likelihood ratio test indicated only one exponential in the open distribution and two in the shut.

Increasing the dead time increased the time constants of the exponential components describing the distributions. The effect is indicated by the shift to the right of the distributions. Fig. 6 plots the time constants of the (detected) exponential components of the open and shut distributions against dead time. The most pronounced effect of missed events for Scheme III is on the exponential component arising from state O_1, since large numbers of the closings to the brief lifetime state C_3 are missed. The time constant of the component arising from O_1 increased from 3.1 ms with no missed events to 35.6 ms when all events <2 ms were missed (Fig. 6 *B*). Compared with this 10-fold increase, the time constant of the exponential component associated with state O_2 increased only 20% (Fig. 6 *A*).

The greatest effect of missed open events on the distribution of shut intervals was on the shut component with intermediate time constant (Fig. 6 *D*, 111% increase). There was less of an effect on the components of short (Fig. 6 *C*, 14% increase) and long (Fig. 6 *E*, 27% increase) time constants. The intermediate component is affected most, since this component is mainly determined by the lifetime of state C_4 (see Magleby and Pallotta, 1983) which is adjacent to open state O_2. State O_2 has a short mean open time, and consequently, many openings to O_2 are missed.

Missed events also had dramatic effects on the areas of the exponential components for the open and shut distributions (Fig. 7). (The area of a component is proportional to the number of events in that component.) For the two open distributions increasing the dead time decreased the area of the open component with the longer time constant and increased the area of the open component with shorter time constant, until a dead time of 1 ms, after which the short component was no longer detected and all the area was in the long open component (Fig. 7 *A*). The areas of the three shut components were only slightly affected by increases in dead time until the shortest component became too brief to detect, after which there were dramatic increases in the areas of the intermediate and longer component (Fig. 7 *B*).

DISCUSSION

Limited time resolution of single channel current recording and analysis results in brief open and shut intervals going undetected. All intervals with durations less than the dead time of the recording and analysis system (see Methods) are missed. When an interval is missed, the intervals immediately preceding and following the missed interval, as well as the missed interval, are detected as a single event with a duration equal to the sum of the durations of the combined intervals. Missed events can produce dramatic increases in mean durations of open and shut intervals which can lead to appreciable errors in estimating underlying kinetic mechanisms if missed events are not taken into account.

This paper uses two different quantitative approaches to determine the effects of missed events on the observed durations of open and shut intervals. The most straightforward, but slowest method is step-by-step simulation of the processes involved in generation of open and shut intervals and their detection. Data obtained with this method should approach an exact solution if a sufficient number of intervals are simulated. For this reason we have used the simulated data to test a less direct, but faster method of correcting for missed events. The second method uses effective rate constants to calculate predicted distributions of open and shut intervals. For durations greater than two times the dead time, results obtained by both methods were within 1% for two-state models (Fig. 3) and agreed within a few percent for models with multiple open and shut states (Fig. 4, Table I), including those with compound states and loops in the kinetic schemes.

Effects of Missed Events

Missed events can have four major effects on observed distributions of open and shut intervals: (*a*) additional components can be added to the distributions of open and shut intervals (Roux and Sauvé, 1985); (*b*) exponential components expected on the basis of the underlying model can be eliminated; (*c*) the time constants of decay of the observed components in the distributions are increased; and (*d*) the areas of the different components can be either increased or decreased.

If more than 40–50% of both open and shut intervals are missed, then the observed distributions of intervals can contain more components than might be expected on the basis of the number of underlying states, assuming one component per state. If fewer events are missed, then

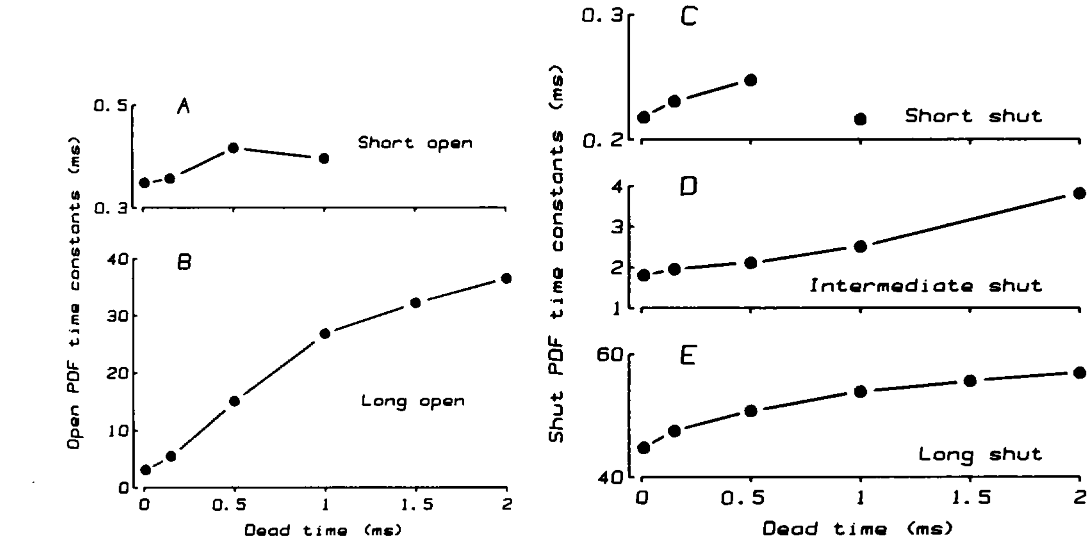


FIGURE 6 Increasing dead time increases the time constants of the exponential components of the observed open (*A*, *B*) and shut (*C–E*) distributions. Time constants were obtained from maximum likelihood fits to data simulated with Scheme III for different dead times. The complete distributions for some of the dead times are presented in Fig. 5. The absence of plotted points for the short open and short shut states after 1 ms dead time indicates that the components were no longer detected. The brief deviated point at 1 ms dead time for the short shut state may arise from phantom states described in Scheme II.

added components are not typically apparent (Figs. 3 and 4). When present, the added components have an effective duration of less than two times the dead time. Thus, fitting the data to only those intervals greater than two times the dead time will prevent identification of components that can arise from phantom states as a consequence of missed events (see Scheme II). With this precaution the number of states should not be overestimated from the number of exponential components (Roux and Sauvé, 1985).

The effect of missed events on distributions of open and shut intervals was examined for a simplified model of the large conductance Ca-activated K channel. As the time resolution was decreased, as would occur with increased filtering, the number of detected events was greatly reduced, the faster exponential components in the distributions were no longer detected, and the time constants of the observed components increased (Figs. 5–7). Since missing events typically changes all components of all distributions, any experimental procedure that selectivity affects only the shortest duration states, and hence has a large effect on the

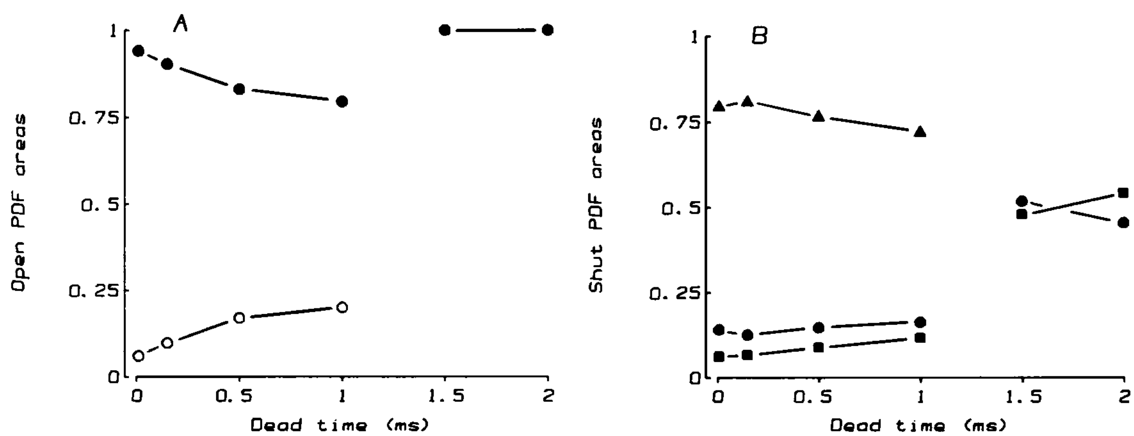


FIGURE 7 Increasing dead time changes the areas of the exponential components of the distributions of open and shut intervals. Areas were obtained from maximum likelihood fits to the same simulated data used for Figs. 5 and 6. (*A*) Areas of long duration open component (filled circles) and short duration open component (open circles) as a function of dead time. Areas indicate the fraction of events in idealized components extending to 0 time. At dead times longer than 1 ms the fast component became undetectable so the area of the slow component becomes 1. (*B*) Areas of short duration (filled triangles), intermediate duration (filled circles), and long duration (filled squares) components of the shut distribution. At dead times greater than 1 ms the short duration component is no longer detected. Therefore, the areas of the intermediate and long component abruptly increase after 1 ms.

numbers of missed events, will affect all the observed distributions and estimated underlying rate constants, if corrections for missed events are not made.

The effect of limited time resolution on data from the large conductance Ca-activated K channel is even more dramatic than shown in Figs. 5–7. The experimental conditions used to determine the rate constants used in Scheme III gave a dead time of ~0.1–0.15 ms (Magleby and Pallotta, 1983). Even this level of filtering was sufficient to exclude faster components not present in Figs. 5–7 (McManus and Magleby, 1985). Since molecular motions in proteins occur over a broad time range extending to rates far faster than can be resolved by patch clamp techniques (Karplus and McCammon, 1981), it might be expected that the number of observed states for a given channel will continue to increase with improvements in time resolution (see, for example, Sigworth, 1985; Lauger, 1985).

Figs. 5–7 show that the errors introduced by not correcting for missed events can be highly significant. Supporting this observation, we have found that the most likely model for a given data set often changes when the data are corrected for missed events (Blatz and Magleby, unpublished observations).

Comparison with other Correction Methods

Rickard (1977) has provided an exact solution for the effect of missed events. The solution is, however, restricted to two-state models with identical open and closing rates, an unlikely situation for biological channels.

Sachs et al. (1982) have presented a simple method to correct a two state model for missed events which neglects the durations of the missed events. For many applications for a two state model the method of Sachs et al. (1982), described by our Eq. 17, can be adequate. For example, their equation predicts the time constants of the observed open and shut intervals for Scheme I when $k_{12} = 1{,}000\ s^{-1}$, $k_{21} = 5{,}000\ s^{-1}$, and the dead time = 0.1 ms with only ~2% error. The error increases to ~5% for symmetrical rates of $1{,}000\ s^{-1}$ and dead time of 1 ms. Errors of this magnitude would be negligible for experiments with limited numbers of events.

Magleby and Pallotta (1983) corrected for the effect of undetected shut events on mean open time from the observed numbers of bursts and captured shut intervals. Their method is restricted to models with only one open state, or to models with two or more open states in which the majority of openings are to only one of the open states.

Colquhoun and Sigworth (1983) have listed equations to correct a two state model for missed events. Our Eqs. 7, 11, and 16 can be transformed into the correction Eqs. 79 and 80 of Colquhoun and Sigworth. Thus, for a two-state model, our correction is identical to theirs.

Neher (1983) has also derived equations to correct for missed events for a two-state model. Effective rate constants and observed lifetimes of open and shut states calculated with his equations and ours typically differ by 3–40% over the conditions we have explored. For example, for the two-state model described by Scheme I ($k_{12} = 1{,}000\ s^{-1}$, $k_{21} = 5{,}000\ s^{-1}$, dead time of 0.1 ms) Neher's equations predict a time constant for the decay of the shut intervals of 0.275 ms compared to the value we obtained from both predicted and simulated data of 0.226 ms, a 22% difference. Differences for the open distribution were less (2.7%).

After our paper was first submitted, a paper by Roux and Sauvé (1985) was published which presents a general formulation for the time interval omission problem and specific examples for the case of channels with one open state. In principle, the method of Roux and Sauvé (1985), which can provide an exact solution, would be preferred to the methods we present. However, because of the numerical complexity of the exact solution of Roux and Sauvé, even for the simplest cases, other methods may still be of use.

To this end Roux and Sauvé present a first-order approximation for their general formulation in which the durations of missed events are neglected. Their first order approximation for a two state model is equivalent to that of Sachs et al. (1982) described by our Eq. 17, and is accurate to within 2–5% for the models we have tested. Whereas their correction based on a first-order approximation for a two-state model would be adequate for many purposes, the simple correction described by Eqs. 79 and 80 in Colquhoun and Sigworth (1983) and our Eq. 16 might be preferred, as it incorporates the duration of missed events, gives an exact solution for the mean duration of observed intervals, has errors typically <1% for observed time constants of decay, and is easily calculated.

Restrictions on the Methods used to Correct for Missed Events

Four or more missed events in a row could appear as a partially conducting state due to the time averaging of the current during the rapid transitions between the brief open and shut intervals. All of the correction methods considered above including ours do not distinguish between apparent partially conducting states due to missed events and actual partially conducting states. For many channels examined, the shortest shut events appear to occur next to the longest open events, and vice versa (Magleby and Pallotta, 1983; McManus, Blatz and Magleby, 1985). Consequently, if the filtering is not excessive, only a small fraction of the missed events should occur consecutively, and these methods should apply.

Except for the exact solutions of Roux and Sauvé (1985) and Rickard (1977), all the considered correction methods are restricted to predicting the distributions of open and shut intervals for durations greater than about two times the dead time, if large numbers of open and shut intervals are missed.

The correction methods of Sachs et al. (1982), Colquhoun and Sigworth (1983) and Neher (1983) are restricted to two state models. The examples presented by Roux and Sauvé (1985) were for models with one open state, but their mathematical framework could be extended to more complicated models. Our method of solving for predicted distributions with effective rate constants and the Q-matrix method of Colquhoun and Hawkes (1981) allows most any model to be corrected, including those with compound states, multiple open and shut states, and loops. Our method is easily formulated as a general computer program that can calculate effective rate constants for these models.

Our method of predicting distributions from effective rate constants is restricted to cases in which the mean lifetimes of the states in the model are sufficiently long that the states would be detected. Since this is the case for models derived from experimental data, this is not a serious restriction. When the dead time becomes greater than the mean lifetime of some of the underlying states, then computer simulation or the approach of Roux and Sauvé (1985) provide means to calculate the expected distribution of open and shut intervals.

Determining Rate Constants from Distributions of Open and Shut Intervals

We determined true rate constants from distributions of open and shut intervals using iterative methods containing a step in which predicted distributions were calculated from effective rate constants. Interestingly, two or more equally likely solutions could be obtained for most models, including those without loops. Depending on the model, one or more of the solutions arose from the effect of missed events. The incorrect solutions from missed events could be identified by analysis of the data with different dead times, as suggested in Colquhoun and Sigworth (1983). This is possible since the true rate constants should be independent of dead time.

Even after excluding the incorrect solutions due to missed events, models with a minimum of three shut and two open states, with or without loops, did not have a single unique solution for the most likely set of rate constants. Models without loops typically had two sets with the relative lifetimes of the two open states reversed. Models with loops could have many equally likely sets of rate constants. Fredkin et al. (1985) have previously indicated that models with loops may not have a unique set of rate constants.

Simultaneous fits to data obtained under several experimental conditions may allow a unique set of rate constants to be determined for a given kinetic scheme. The correction methods of Roux and Sauvé (1985) could also be used in iterative procedures to obtain estimates of underlying rate constants. They further suggest that an iterative curve fitting procedure focused mainly on how the different observed time constants vary with dead time may allow more accurate estimation of transition rates.

In summary, we present a method to correct for missed events which can predict with reasonable accuracy the observed distributions for a given time resolution, kinetic scheme, and rate constants. Our method can also be used with iterative techniques to determine rate constants consistent with the data from observed distributions for a given kinetic scheme and time resolution.

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REFERENCES

Clay, J. R., and L. J. DeFelice. 1983. Relationship between membrane excitability and single channel open-close recording. *Biophys. J.* 42:151–157.

Colquhoun, D. 1971. Lectures on Biostatistics. Clarendon Press, Oxford, England. 263.

Colquhoun, D., and A. G. Hawkes. 1981. On the stochastic properties of single ion channels. *Proc. R. Soc. Lond. B. Biol. Sci.* 211:205–235.

Colquhoun, D., and A. G. Hawkes. 1983. The principles of the stochastic interpretation of ion-channel mechanisms. *In* Single-Channel Recording. B. Sakmann and E. Neher, editors. Plenum Publishing Corp., New York and London. 135–175.

Colquhoun, D., and B. Sakmann. 1981. Fluctuations in the microsecond time range of the current through single acetylcholine receptor ion channels. *Nature (Lond.).* 294:464–466.

Colquhoun, D., and F. J. Sigworth. 1983. Fitting and statistical analysis of single-channel records. *In* Single-Channel Recording, B. Sakmann and E. Neher, editors. Plenum Publishing Corp., New York and London. 191–263.

Cox, D. R., and H. D. Miller. 1965. The Theory of Stochastic Processes. Methuen and Co., Ltd., London. 7.

Finkelstein, A., and C. S. Peskin. 1984. Some unexpected consequences of a simple physical mechanism for voltage-dependent gating in biological membranes. *Biophys. J.* 46:549–558.

Fredkin, D. R., M. Montal, and J. A. Rice. 1985. Identification of aggregated markovian models: application to the nicotinic acetylcholine receptor. *In* Proceedings of the Berkeley Conference in Honor of Jerzy Neyman and Jack Kiefer. Wadsworth Publishing Co., Belmont, CA.

Hamill, O. P., A. Marty, E. Neher, B. Sakmann, and F. J. Sigworth. 1981. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pfluegers Arch. Eur. J. Physiol.* 391:85–100.

Hille, B. 1984. *Ionic Channels of Excitable Membranes.* Sinauer Associates, Sunderland, MA. 426 pp.

Horn, R., and K. Lange. 1983. Estimating kinetic constants from single channel data. *Biophys. J.* 43:207–223.

Karplus, M., and J. A. McCammon. 1981. The internal dynamics of globular proteins. *CRC Crit. Rev. Biochem.* 9:293–349.

Labarca, P., J. A. Rice, D. R. Fredkin, and M. Montal. 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.* 47:581–591.

Magleby, K. L., and B. S. Pallotta. 1983. Calcium dependence of open and shut interval distributions from calcium-activated potassium channels in cultured rat muscle. *J. Physiol. (Lond.).* 344:585–604.

McManus, O. B., A. L. Blatz, and K. L. Magleby. 1985. Inverse relationship of the durations of adjacent open and shut intervals for Cl and K channels. *Nature (Lond.)*. 317:625–627.

McManus, O. B., and K. L. Magleby. 1985. The large conductance calcium-activated potassium channel in cultured rat muscle has at least three open states of similar conductance and six shut states. *Biophys. J.* 47(2, pt.2):137*a*. (Abstr.)

Methfessel, C., and G. Boheim. 1982. The gating of single calcium-dependent potassium channels is described by an activation/blockade mechanism. *Biophys. Struct. Mech.* 9:35–60.

Miller, C. 1983. Integral membrane channels: studies in model membranes. *Physiological Rev.* 63:1209–1242.

Moczydlowski, E., and R. Latorre. 1983. Gating kinetics of Ca^{2+}-activated K^+ channels from rat muscle incorporated into planar lipid bilayers: evidence for two voltage-dependent Ca^{2+} binding reactions. *J. Gen. Physiol.* 82:511–542.

Neher, E. 1983. The charge carried by single-channel currents of rat cultured muscle cells in the presence of local anaesthetics. *J. Physiol. (Lond.)*. 339:663–678.

Pallotta, B. S. 1985. Calcium-activated potassium channels inactivate from a short-duration open state. *Biophys. J.* 47(2, pt.2):135*a*. (Abstr.)

Rickard, J. T. 1977. The zero-crossing interval statistics of the smoothed random telegraph signal. *Information Sciences*. 13:253–268.

Roux, B., and R. Sauvé. 1985. A general solution to the time interval omission problem applied to single channel analysis. *Biophys. J.* 48:149–158.

Sachs, F., J. Neil, and N. Barkakati. 1982. The automated analysis of data from single ionic channels. *Pfluegers Arch. Eur. J. Physiol.* 395:331–340.

Sigworth, F. J. 1985. Open channel noise. I. Noise in acetylcholine receptor currents suggests conformational fluctuations. *Biophys. J.* 47:709–720.